“Missing microbes” in commercial broiler production and the effects of early-life microbial inoculations on broiler microbiota development

by

Camila Schultz Marcolla

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

ABSTRACT

Modern poultry production relies on strict biosecurity procedures to minimize the risk of introducing pathogens to flocks, and may inadvertently limit exposure to beneficial commensal bacteria that naturally coexist with chickens. Commensal bacteria promote gastrointestinal and immune development, nutrient metabolism, and disease resistance. Consequently, broilers raised without a proper commensal microbiota may be more susceptible to diseases, exhibit abnormal immune responses, and have limited growth potential. Although recent advancements in sequencing technologies and bioinformatics have enhanced our understanding of broiler gut microbiota, most research is still limited to taxonomical descriptions and correlational findings, which alone cannot uncover specific bacterial functions or the mechanisms underlying observed effects on host physiology. In addition, studies have primarily focused on broilers in intensive systems and experimental facilities, potentially failing to represent the microbiota of a "normal" chicken, that would naturally hatch in a nest and be readily colonized by a mature microbiota from hens. Given that the gut microbiota contributes significantly to host health and that coevolution shapes host-microbe relationships to be beneficial, it is reasonable to expect that a mature hen's microbiota would more accurately represent a normal, healthy, and stable microbiota, than that of an intensively raised broilers. Therefore, we hypothesize that intensive farming practices limit broilers' exposure to coevolved bacteria that would typically be present in the chicken gut under more natural circumstances. Additionally, we hypothesize that early-life exposure to chicken commensal bacteria can modulate broiler immune responses and disease resistance, and that coevolved native bacteria possess the ability to efficiently colonize the chicken gut after a single exposure.

The first study characterized the cecal microbiota of 35-day-old broilers from intensive production systems (IPS) and from extensive production systems (EPS) on commercial farms in Alberta. We aimed to identify the core microbiota of broiler ceca and determine which bacteria were absent in IPS broilers. We found that the microbiota of broilers in EPS had higher phylogenetic diversity and greater predicted functional potential compared to IPS. Additionally, bacterial taxa ubiquitous in EPS microbiota, such as *Olsenella*, *Alistipes*, *Bacteroides*, *Barnesiella*, *Parabacteroides*, *Megamonas*, and *Parasutterella* were infrequent or absent in the microbiota of IPS broilers. Additionally, we collected and identified 410 bacterial isolates, representing 87 unique species, that can be used as a resource in future studies.

The second study evaluated the impact of different microbial preparations, inoculation strategies, and inoculum sources on the gut microbiota and physiological responses of broilers. We found that chicks exposed to cecal contents or microbial cultures were readily colonized by Bacteroidetes and showed higher abundance of *Alistipes*, *Bacteroides*, *Barnesiella*, *Mediterranea*, *Megamonas*, *Parabacteroides*, *Phascolarctobacterium* and *Subdoligranulum* compared to control birds without microbial exposure. We also found that gavage, spray, and cohousing methods were effective to promote colonization, and that all microbial preparations promoted a reduction in the relative abundance of *Escherichia-Shigella* in exposed birds.

The third study evaluated the effect of early-life introduction of *M. hypermegale* alone or in combination with a defined community (DC) of bacteria on broiler gut microbiota development and ability to resist *Salmonella* infection. Substantial changes in cecal microbiota composition were observed with the introduction of the DC, but effects on host physiology and *Salmonella* resistance were moderate. We identified *A. finegoldii*, *B. gallinaceum*, *B. viscericola*, *P. vulgatus*, *L. crispatus*, and *L. agilis* as good colonizers of the chicken gut. Moreover, the introduced bacteria

caused a reduction in the relative abundance of *Escherichia-Shigella*, which was consistent with previous findings.

In summary, broilers in IPS exhibited lower abundance of core microbes and putative functions in their cecal microbiota compared to broilers in EPS. We identify bacterial lineages that were reduced in IPS but were successful colonizer in birds exposed to complex or defined communities, suggesting these are host-adapted microbes that have had their dispersal among broilers negatively affected by current production practices. Despite the significant effects of microbial preparations on the microbiota community of inoculated birds, the effects on measured host responses were moderate. The collection of bacterial isolates generated in this study is a valuable resource for future research.

PREFACE

This thesis is an original work by Camila Schultz Marcolla. The research projects included in this thesis have been approved by the University of Alberta Research Ethics Board under Pro00071050, AUP00002373, AUP00002572 and AUP00001626.

The literature review in Chapter 2 has been published as Camila Schultz Marcolla, Carla Souza Alvarado, Benjamin P. Willing. 2019. Early-life microbial exposure shapes subsequent animal health. Can. J. Anim. Sci. 99:661-677. B.P. Willing conceived the research and edited the manuscript. C.S. Marcolla and C. S. Alvarado wrote and edit the manuscript.

The study in Chapter 3 was designed by B. P. Willing and C. S. Marcolla. C. S. Marcolla recruited poultry producers to participate in the study, collected samples, performed 16S rRNA sequencing analysis, analyzed data, interpreted the results, performed bacterial isolation and characterization, and wrote the manuscript. T. Ju performed sample collection and analysis, analyzed whole genome sequencing data, and edited the manuscript. H. L. Lantz performed bacterial isolation and characterization. B. P Willing edited the manuscript. C. S. Marcolla and T. Ju contributed equally to this work. The manuscript is accepted for publication in Microbiology Spectrum as Camila Schultz Marcolla, Tingting Ju, Hannah L. Lantz, Benjamin P. Willing. 2023. Investigating the cecal microbiota of broilers raised in extensive and intensive production systems.

The study in Chapter 4 has been published as Camila S. Marcolla, Tingting Ju, Benjamin P. Willing. 2023. Cecal microbiota development and physiological responses of broilers following early life microbial inoculation using different delivery methods and microbial sources. Applied Environmental Microbiology 89(5):e0027123. B.P. Willing conceived the research and edited the manuscript. C.S. Marcolla designed and performed the experiments, analyzed the data, interpreted the results, and wrote the manuscript. T. Ju edited the manuscript.

The study in Chapter 5 is in preparation as Camila Schultz Marcolla, Tingting Ju, Kimberlee Ten, Benjamin P. Willing. 2023. Impact of a defined bacterial community and *Megamonas hypermegale* on broiler microbiota and resistance to *Salmonella* infection. C. S. Marcolla designed the experiment, collected and analyzed data, interpreted the results, and wrote the manuscript. T. Ju performed sample collection, analyzed data, and edited the manuscript. K. Lee performed *in vitro* experiments with *M. hypermegale*. B. P. Willing edited the manuscript.

ACKNOWLEDGMENTS

I would like to express my heartfelt gratitude to Dr. Benjamin P. Willing for providing me with the opportunity to conduct this research and for fostering my independence as a researcher. His support and guidance throughout this journey have been instrumental to its success. I extend my appreciation to Dr. Leluo Guan and Dr. Daniel Barreda for their contributions as members of my supervisory committee, and to Dr. Jennifer Ronholm and Dr. Anne Laarman for their insightful advice as examining committee members. I am indebted to Dr. Tingting Ju, for her constant guidance on laboratory procedures and data analysis. Their expertise and mentorship have been indispensable in navigating the complexities of this research.

I am grateful to the poultry producers that collaborated with this project and to the Alberta Egg Producers, Alberta Chicken Producers and Poultry Health Services. I acknowledge the support from Science Animal Support Services, particularly Sarah Collard, and Health Sciences Laboratory Animal Services, especially Kaiti Valm, during animal experiments, and Stephanie Tollenaar for her assistance with laboratory and histology analysis.

I sincerely appreciate the support of the members of the Willing lab, past and present, for their support. Special thanks to Deanna Pepin, Dr. Janelle Fouhse, Dr. Andrew Forgie, Carla Sosa Alvorado, Dr. Tingting Ju, and Hannah Lantz. Tingting and Hannah, you have been exceptional researchers, teachers, and individuals who have continuously inspired and supported me throughout this journey. I am glad I had you two by my side as I knocked over piles of freshly poured agar plates and pipetted samples with my plate upside down.

I am grateful to the Department of Agricultural, Food and Nutritional Science, the Faculty of Graduate Studies and Research at the University of Alberta, Alberta Innovates and Canadian Poultry Research Council for their support and generous funding.

Finally, I would like to thank my parents, who had embraced and supported every decision I have made, and my sister, and my niece Ana Laura, for their love and support. I also would like to thank my partner, Levi, for being the bedrock of my journey, for his companionship and dedication to our family. Last, I would like to thank Mr. Antonio, for imparting upon me the invaluable lesson that this degree is not as important, nor that challenging, nor that rewarding as I previously thought it could be, and for showing me that, although this research is interesting, it will never be more inspiring or relevant than rocks and pinecones. These are way more fascinating.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE: aerobic

AN: anaerobic

ASV: amplicon sequence variant

BW: body weight

DC: defined community

DESeq2: Differential gene expression analysis based on the negative binomial distribution

EPS: extensive production systems

IFN: interferon

IL: interleukin

IPS: intensive production systems

LCY: liquid casein yeast

M-CSF: macrophage colony-stimulating factor

MIP: macrophage inflammatory protein

PBS: phosphate buffered saline

PCoA: Principal coordinates analysis

PCR: polymerase chain reaction

PD: Phylogenetic diversity

PICRUST: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

qPCR: quantitative polymerase chain reaction

RANTES: CCL5/regulated on activation, normal T cell expressed and secreted

rRNA: ribosomal ribonucleic acid

SCFA: short-chain fatty acids

SD: standard deviation

VEGF: vascular endothelial growth factor

CHAPTER 1: INTRODUCTION

High-throughput sequencing methods have revolutionized our understanding of the poultry gut microbiota by offering a rapid and cost-effective approach to characterizing microbial communities without requiring laborious bacterial culturing procedures [1]. These technologies have been helpful to elucidate how host, environmental factors, and diet can impact the poultry gut microbiota [2, 3]. Studies have shown that the gut microbiota plays a major influence on intestinal and immune development [4], and that differences in gut microbiota composition are associated with the occurrence of immune-mediated, inflammatory, metabolic, and neurological diseases [5, 6]. It is suggested that reducing bacterial dispersal among individuals, especially mothers and offspring, can lead to disruptions in microbiota stability, which are linked to higher susceptibility to metabolic, inflammatory, and infectious diseases [7, 8].

The intensive production practices in the poultry industry are structured to prevent the introduction and spread of infectious diseases among poultry flocks, but can inadvertently reduce the dispersal of gut bacteria that might be of importance for chicken health and physiology. It has been consistently demonstrated that artificially hatched chicks are susceptible to *Salmonella* infection and that colonization resistance can be conferred by inoculating chicks with cecal contents from adult chickens [9–11]. More importantly, and usually overlooked, is the fact that the microbiota harvested from other species, such as horses and cows, and inoculated to chicks do not confer any level of protection [12]. This illustrates how the establishment of a gut microbial community that is stable and host-adapted can have major impacts on animal health. In this review, we discuss intensive production practices in the broiler industry, how they hinder the establishment of commensal microbial communities, and what are the potential consequences of these disruptions in microbiota assembly.

1.1.1. The poultry industry and its potential impact on the chicken intestinal microbiota

It is generally accepted that the domestic chicken originated from the junglefowl between 50,000 and 125,000 years ago, and was domesticated at about 8,000 years ago [13]. Roman documents indicate the existence of a rudimentary poultry industry structure as far back as 2,000 years ago, with guidelines regarding chicken housing, feeding, breeding, and disease control [14]. Around the 1920s, chickens began to be selectively bred for meat or egg production, originating the broiler and layer industries. Subsequently, integrated operations and specialized breeding farms emerged, resulting in rapid advancements in poultry farming [15]. Over the last 50 years, the poultry industry has experienced a significant transformation, driven by the implementation of scientific knowledge and cutting-edge technologies. This continuous progress has positioned the broiler industry as the leading provider of meat worldwide [16].

As the poultry industry evolved, chickens were moved from the outdoors into environmentally controlled indoor barns. This facilitates animal husbandry, increases production efficiency, and protects the birds from predators; however, the high animal density favors the emergence and spread of infectious diseases [17]. Antibiotics, which were initially used to treat and prevent bacterial infections, were observed to promote higher growth rates in chicks [18], and introduced in feed formulations as means to improve animal growth, production efficiency, and prevent diseases. The nutrient requirements of broilers were established, and precisely formulated diets containing a limited number of high-quality ingredients were introduced. Also, a complete separation between parental flocks and their offspring was established. Fertilized eggs are collected soon after laid and transported to sanitized hatcheries. The artificially hatched chicks are transported to empty barns and housed with chicks of the same age. Although these measures

reduce the occurrence of infectious diseases, they also minimize chicks' exposure to healthy commensal bacteria that had likely co-evolved with chickens for the past 50,000 years.

The gut microbiota is the community of microorganisms living in the gastrointestinal tract of animals and these bacteria have major effects on host physiology and disease resistance. The chicken gut microbiota promotes immune [19–22] and gastrointestinal development [23–28], and reduces the establishment of pathogenic bacteria [9, 10]. On the other hand, the chicken has a metabolic cost associated with housing, maintaining, and managing the microbiota community [29]. Manipulating the gut microbiota to enhance production performance and disease resistance represents a significant challenge in broiler production. In recent years, there has been a surge in studies that provide descriptive and correlational information on how various factors influence the composition of the microbiota. However, we still lack comprehensive mechanistic insights and a fundamental understanding of the specific functions exerted by individual bacteria and complex microbial communities in broiler physiology.

1.1.2. Microbiota assembly in intensive broiler production

Microbiota assembly refers to the establishment and development of the bacterial community in the gut. Assemblage is variable and influenced by three main processes, which can be stochastic, deterministic, and historical [30]. Stochastic processes refer to random events, such as the chance that a chicken will encounter a microbe and the chance that the encountered microbe will colonize; however, the chance that a microbe will colonize, or not, also depends on deterministic and historical processes. Deterministic processes refer to specific factors that affect microbiota assembly in a non-random manner [31], for example, the ability of the bacteria to withstand the pH, oxygen levels, and peristalsis within the gastrointestinal tract. Nonetheless,

bacteria that are adapted to the gastrointestinal tract still have their colonization ability affected by historical contingency, which refers to the presence of previously established bacteria that can impede or aid the establishment of a new species [30].

Our current understanding of microbiota assembly processes in the broiler gut is limited to descriptions of succession patterns of community composition as birds age. Although microbiota composition reported across studies varies, there is a level of consensus about microbial succession patterns in broilers [32–35], which are usually described as three phases. First, the cecal microbiota at hatch is dominated by Proteobacteria, especially *Escherichia-Shigella*, and *Lactobacillales*, mainly *Streptococcus*, are also reported [36–39]. The second stage is characterized by the establishment of Firmicutes, mainly Clostridia, such as *Lachnospiraceae* and *Ruminococcaceae*, *Candidatus Arthrobacter* and *Romboutsia*, which occurs around 4 to 7 days old [36, 37]. In addition, Bacteroidetes establish between 10 to 14 days, including the genera *Alistipes*, and families *Prevotellaceae* and *Porphyromonadaceae* [36, 39]. The last stage occurs around three weeks of age, when Firmicutes, such as *Oscillospira* and members of the *Veillonellaceae* family will establish [36]. By 21 days of age, the microbiota is considered to be mature and is usually dominated by Firmicutes, Bacteroidetes and Proteobacteria.

The microbial successional patterns of artificially reared chickens are substantially different from those observed in chickens that had access to a mature chicken microbiota. Most studies indicate that the microbiota of week-old broilers is dominated by Firmicutes and Proteobacteria [40, 41], however, once birds are exposed to a mature chicken microbiota, Firmicutes and Bacteroidetes are the dominant phyla. This pattern was observed in chickens co-housed with a hen [40], inoculated with cecal contents or cultures [41, 42], exposed to used litter [43], inoculated with defined communities (Chapter 5), and with access to an outdoor range [44].

While Bacteroidetes seem to readily colonize and expand in chickens exposed to mature microbiota, some studies indicate the relative abundance of Bacteroidetes to be less than 10% of the community in 35-day-old broilers reared in intensive systems and experimental facilities [45, 46].

In natural conditions, chicks hatch in nests containing a mix of plant material, feathers, and fecal content from the broody hen. After hatching, chicks stay with the hen for at least 6 weeks, and forage on almost anything, including foliage, grains, seeds, invertebrates, worms, and small vertebrates [47]. In natural conditions, chicks are exposed to a large array of maternal and environmental microbes since birth. As the gut microbiota influences digestion and nutrient absorption, promotes immune system development, intestinal integrity, and nutrient synthesis, it is expected that chickens that harbor a beneficial microbiota are more likely to obtain nutrients, overcome challenges, grow, reproduce, and transmit their commensal microbes across generations [48–50]. On the other hand, broiler chicks hatched in sanitized incubators are likely to be colonized by environmental and human bacteria, and to present delayed and inconsistent microbiota development compared to "normal chickens", consequently, the absence of "chicken microbes" in our production systems is likely to impact broiler microbiota function, physiology, and the ability to ward off pathogenic bacteria.

1.1.3. Competitive exclusion

Broiler chicks with no access to a mature microbiota were shown to be more susceptible to *Salmonella* infection and considered to be "*in a transitional state between germ-free and normal animals for a few days*" [9]. This has been consistently demonstrated by numerous studies [51–64]. This led to the development of the "competitive exclusion (CE) of pathogens"

concept, which describes the ability of a microbial community to outcompete pathogens for nutrients and attachment sites within the gut, and to produce antibacterial substances that hinder pathogen establishment [65]. Competitive exclusion is also referred to as “microbial barrier” and “colonization resistance” [66], although the latter also includes the effects of bacteria modulating host immune barrier and responses [67]. Several CE products containing combinations of selected intestinal bacteria from poultry have been developed and employed to successfully control *Salmonella* occurrence in some countries [10, 65]. However, CE products present inconsistent results and the level of protection against pathogenic bacteria is still inferior to that promoted by the inoculation of cecal contents or undefined cultures [11].

Over the course of more than 60 years, numerous studies have consistently shown that commensal bacteria can protect chickens from pathogenic infections. Lev and Forbes (1958) [68] have challenged germ-free chickens with *Clostridiodes difficile* and observed a 17% reduction in body weight. In a subsequent experiment, germ-free chickens were colonized with three commensal bacteria - *Streptococcus liquefaciens*, *Lactobacillus lactis* and *Escherichia coli* - harvested from poultry intestinal contents. The growth response in these pre-colonized birds was comparable to that of germ-free chickens that were not challenged with *C. difficile*. The pre-colonized birds exhibited improved body weight and lower *C. difficile* loads compared to germ-free chickens challenged with *C. difficile* [68].

These findings indicate that commensal bacteria have the ability to colonize the gut without causing detrimental effects and that this colonization reduces the burden caused by potential pathogens. However, the specific mechanisms underlying the protective effect remain to be fully elucidated. Additionally, further research is needed to determine the specific bacteria, or bacterial functions, necessary to achieve such protection.

1.1.4. Community stability

Resistance is one of the drivers of microbiota community stability. It refers to the ability of the microbiota to resist disturbances that can alter community composition and function [69]. Disturbances of the microbial communities can be caused by numerous factors. It was demonstrated that the poultry gut microbiota composition changes in response to a plethora of interventions, including nutrient levels, feed additives, antibiotics, pathogen infection, intestinal inflammation, toxins, probiotics, microbial inoculations, vaccinations, stress, housing, and others [70-78].

Disruptions in the composition, diversity, and function of the microbiota community can lead to dysbiosis. Dysbiosis is broadly defined as an unstable microbial community [79, 80] caused by the loss of beneficial organisms and/or the overgrowth of harmful bacteria [81]. Dysbiosis can lead to functional changes in the microbiota, inflammation, infections, and production losses.

Another component of microbiota community stability is resilience. Community resilience is broadly defined as the rate at which a microbiota community can recover after being disturbed and return to the pre-disturbance state [79]. A stable community is also one that is dynamic and able to respond and adapt to environmental situations [82]. The presence of multiple species or groups of bacteria within the gut microbiota that performs similar functions contributes to microbiota stability because if one species is unable to carry out a particular function, another species can compensate for it, thus, functional redundancy ensures that essential functions are maintained even in face of microbiota perturbations and loss of keystone species [82–84].

In nature, animals experience changes in environmental conditions, predation, disease occurrence, and fluctuation in food sources that are usually suboptimal for growth and

reproduction. It is likely that a native microbiota community is shaped to endure these disruptions and efficiently return to a steady state. In fact, the loss of native microbiota is considered one of the factors that hinder the survival of captive animals upon re-introduction to their native environments [85]. For example, *Synergistetes* bacteria can degrade toxic compounds from plants and were shown to be present in the ceca of wild capercaillie birds, but absent in captive animals. The absence of *Synergistetes* impairs the ability of captive birds to feed on phenol-rich needles from coniferous plants, which are the only food source available during the winter in their native environment, thus reducing the survival of captive birds upon reintroduction to wild environments [86], and highlighting the importance of co-evolved microbes.

1.1.5. Coevolution

Microbiota co-evolution refers to the reciprocal evolutionary adaptations between host organisms and their microbial communities. Co-evolution is likely to favour ecological relationships that are mutually beneficial. Bacteria that can effectively colonize the gut without triggering extensive immune reactions, that produce useful compounds, such as vitamins and short-chain fatty acids, and that are capable of outcompeting pathogens for substrates and attachment sites, can improve host fitness and survival. In exchange, hosts provide bacteria with shelter and a constant flow of nutrients, also promoting bacterial survival by transmitting the commensal microbes to the next generation [31, 49, 87].

Bacteria that co-evolved with hosts are likely to be highly adapted to the gut environment and to contribute with important functions to the host. A study has shown that the microbiota of chickens inoculated with a mix of *Ligilactobacillus reuteri* containing strains harvested from chickens, rodents, humans, and pigs, is dominated by the strain that originated from chickens.

Similarly, the microbiota of mice inoculated with the same mix will be dominated by the strain that originated from a mouse [88]. Moreover, *L. reuteri* strains were only able to form biofilms when introduced to their respective hosts [89]. Another study demonstrated that *Bacteroides thetaiotaomicron* regulates the synthesis of gut epithelial glycans and use these glycans as substrates, thus modifying the gut environment in their favor [90].

Not only do host-adapted microbes present mechanisms to adapt themselves to their hosts, but they also develop mechanisms to help their hosts adapt to the surrounding environment. As an example, *Phocaeicola plebeius* originating from the gut of Japanese humans was shown to present enzymes capable of degrading carbohydrates from algae, which were absent in *P. plebeius* of North Americans, indicating this bacterium evolved to aid the digestion of seaweed which is part of the daily diet in Japan [91]. Also, bacteria in the gut of wild birds are capable of degrading toxic compounds present in the plants they forage on [86, 92]. Due to their short generation times, bacteria genomes can respond quicker than host genomes to environmental changes and favor host acclimation and adaptation to new situations [93–95].

Animals co-exist and co-evolve with their gut microbiota, mostly for the benefit of bacteria and hosts alike, although these benefits are not free of charge. For the host, there is a metabolic cost associated with housing bacteria. Studies comparing germ-free and conventional chickens have shown that conventional animals grow slower and have lower feed efficiency than germ-free counterparts [23, 28], which is likely the result of the higher rates of intestinal and immune development promoted by bacterial presence [23, 25–28], which culminates with higher rates of protein synthesis in the liver and in the gastrointestinal tract [29].

Historically, the metabolic costs of microbial presence in broiler production were partially mitigated by biosecurity procedures and the use of growth promoter antibiotics (AGPs). The exact

mechanisms by which AGPs exert their growth-promoting effects are not fully understood, but they were shown to influence the gut microbiota and modulate nutrient absorption, metabolism, and immune function in birds [96–99]. Due to concerns regarding the development of antimicrobial resistance, the use of AGP has been banned in many countries, which left the poultry industry scrambling in search of alternatives.

The AGP ban caused a surge in the development and testing of probiotics as alternatives to improve performance and disease resistance in broilers [100, 101]. However, currently, the bacterial strains approved and included as probiotic poultry products are usually harvested from soil and fermented food [102] and are unlikely to effectively colonize and persist in the poultry gut environment unless constantly provided [103]. Also, most strains approved as probiotics for poultry are Firmicutes, especially bacillus and lactobacillus, which are spore-former and aerotolerant [102] and likely to lack mechanisms to persist within the poultry gut [103].

To manipulate broiler microbiota towards improved production and health, it is necessary to account for ecological relationships and to develop a mechanistic understanding of how bacteria and broilers interact and the consequences of these interactions. Microbial colonization in early life is critical for host gastrointestinal and immune development, and disruptions of microbial community stability impact host physiology at later stages of life. The intensive production practices employed in the industry had a major impact on the composition of the broiler gut microbiota, making it substantially different from the microbiota found in a “normal chicken” that lives in a more natural environment. Understanding these differences and elucidating the role of commensal bacteria as individuals and within the community is necessary to develop effective strategies to manipulate the broiler microbiota. In the next chapter, I review how farming practices

influence microbial composition and the potential effects on host physiology, metabolism, and disease resistance

1.2. HYPOTHESIS AND OBJECTIVES

This thesis aimed to generate foundational knowledge and resources to harness the potential of the microbiota in poultry production by determining the variation in cecal microbiota composition between broilers reared in intensive and extensive commercial farms, creating a library of commensal microbes isolated from the chicken gastrointestinal tract, and evaluating the impacts of early-life inoculation of microbial communities and selected bacterial isolates on host responses and microbiota composition.

1.2.1 Hypotheses

1. Intensive farming practices cause insufficient exposure of broilers to chicken commensal bacteria that are found in broilers reared under extensive farming practices
2. Exposure to chicken microbiota favors gut colonization by bacteria that are adapted to the chicken gut and modulate gut health and immune responses

1.2.2 Objectives

1. To characterize the cecal microbiota community composition of broilers raised in commercial farms in Alberta
2. To compare the cecal microbiota of broilers raised in intensive and extensive farming systems and determine if bacteria are missing from broilers from intensive systems
3. To create a culture collection of chicken commensal bacteria
4. To investigate the impact of early-life microbial exposures on gut microbiota development and host responses

5. To evaluate the impact of early-life exposure to a defined community of bacteria and *Megamonas hypermegale* on broiler microbiota development, immune responses and resistance to *Salmonella* infection

1.3. REFERENCES

1. Lee MD, Ipharraguerre IR, Arsenault RJ, Lyte M, Lyte JM, Humphrey B, et al. Informal nutrition symposium: leveraging the microbiome (and the metabolome) for poultry production. Poult Sci. 2022;101:101588.

2. Yeoman CJ, Chia N, Jeraldo P, Sipos M, Goldenfeld ND, White BA. The microbiome of the chicken gastrointestinal tract. Anim Health Res Rev. 2012;13:89–99.

3. Pan D, Yu Z. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 2014;5:108–19.

4. Yeoman CJ, Chia N, Yildirim S, Berg Miller ME, Kent A, Stumpf R, et al. Towards an evolutionary model of animal-associated microbiomes. Entropy. 2011;13:570–94.

5. Lazar V, Ditu L-M, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, et al. Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer. Front Immunol. 2018;9.

6. Arrieta MC, Stiemsma LT, Amenyogbe N, Brown E, Finlay B. The intestinal microbiome in early life: Health and disease. Frontiers in Immunology. 2014;5 AUG.

7. Martínez I, Stegen JC, Maldonado-Gómez MX, Eren MA, Siba PM, Greenhill AR, et al. The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes. Cell Rep. 2015;11:527–38.

8. Ríos-Covian D, Langella P, Martín R. From Short- to Long-Term Effects of C-Section Delivery on Microbiome Establishment and Host Health. Microorganisms. 2021;9:2122.

9. Nurmi E, Rantala M. New aspects of salmonella infection in broiler production. Nature. 1973;241:210–1.

10. Schneitz C. Competitive exclusion in poultry - 30 years of research. Food Control. 2005;16 8 SPEC. ISS.:657–67.

11. Kerr AK, Farrar AM, Waddell LA, Wilkins W, Wilhelm BJ, Bucher O, et al. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on Salmonella spp. prevalence and concentration in broiler chickens. Prev Vet Med. 2013;111:112–25.

12. Rantala M, Nurmi E. Prevention of the growth of *Salmonella infantis* in chicks by the flora of the alimentary tract of chickens. Br Poult Sci. 1973;14:627–30.

13. Lawal RA, Hanotte O. Domestic chicken diversity: Origin, distribution, and adaptation. Animal Genetics. 2021;52:385–94.

14. Wood-Gush DGM. A History of the Domestic Chicken from Antiquity to the 19th Century.

15. The National Chicken Council.

16. OECD. 2022.

17. Gilbert M, Xiao X, Robinson TP. Intensifying poultry production systems and the emergence of avian influenza in China: A “One Health/Ecohealth” epitome. Archives of Public Health. 2017;75.

18. Moore PR, Evenson A, Luckey TD, Mccoy E, Elvehjem CA, Hart EB. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick*.

19. Parry SH, Allen WD, Porter P. Intestinal immune response to E. coil antigens in the germ-free chicken. 1977.

20. Honjo K, Hagiwara T, Itoh K, Takahashi E, Hirota Y. Immunohistochemical Analysis of Tissue Distribution of B and T Cells in Germfree and Conventional Chickens. Journal of Veterinary Medical Science. 1993;55:1031–4.

21. Dascalu S, Preston SG, Dixon RJ, Flammer PG, Fiddaman S, Boyd A, et al. The influences of microbial colonisation and germ-free status on the chicken TCRβ repertoire. Front Immunol. 2023;13.

22. Saint-Martin V, Quéré P, Trapp S, Guabiraba R. Uncovering the core principles of the gut-lung axis to enhance innate immunity in the chicken. 2022. https://doi.org/10.3389/fimmu.2022.956670ï.

23. Yokota H, Coates ME. The uptake of nutrients from the small intestine of gnotobiotic and conventional chicks. British Journal of Nutrition. 1982;47:349–56.

24. Furuse M, Okumura J-I. REVIEW Nutritional and physiological characteristics in germ-free chickens. 1994.

25. Cowieson AJ. Comparative biology of germ-free and conventional poultry. Poultry Science. 2022;101.

26. Cheled-Shoval SL, Gamage NSW, Amit-Romach E, Forder R, Marshal J, Van Kessel A, et al. Differences in intestinal mucin dynamics between germ-free and conventionally reared chickens after mannan-oligosaccharide supplementation. Poult Sci. 2014;93:636–44.

27. Furuse M, Yokota H. Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. British Journal of Nutrition. 1984;51:255.

28. Coates ME, Fuller R, Harrison GF, Lev M, Suffolk SF. A comparision of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. British Journal of Nutrition. 1963;17:141–50.

29. Muramatsu T, Takasu O, Furuse M, Tasaki I, Okumura J. Influence of the gut microflora on protein synthesis in tissues and in the whole body of chicks. Biochemical Journal. 1987;246:475–9.

30. Jones EW, Carlson JM, Sivak DA, Ludington WB. Stochastic microbiome assembly depends on context. Proceedings of the National Academy of Sciences. 2022;119.

31. Walter J, Britton RA, Roos S. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the Lactobacillus reuteri paradigm. Proc Natl Acad Sci U S A. 2011;108 SUPPL. 1:4645–52.

32. Lu J, Idris U, Harmon B, Hofacre C, Maurer JJ, Lee MD. Diversity and Succession of the Intestinal Bacterial Community of the Maturing Broiler Chicken. Appl Environ Microbiol. 2003;69:6816–24.

33. Torok VA, Hughes RJ, Ophel-Keller K, Ali M, MacAlpine R. Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult Sci. 2009;88:2474–81.

34. Johnson TJ, Youmans BP, Noll S, Cardona C, Evans NP, Peter Karnezos T, et al. A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl Environ Microbiol. 2018;84.

35. Torok VA, Allison GE, Percy NJ, Ophel-Keller K, Hughes RJ. Influence of Antimicrobial Feed Additives on Broiler Commensal Posthatch Gut Microbiota Development and Performance. Appl Environ Microbiol. 2011;77:3380–90.

36. Rychlik I. Composition and function of chicken gut microbiota. Animals. 2020;10.

37. Jurburg SD, Brouwer MSM, Ceccarelli D, van der Goot J, Jansman AJM, Bossers A. Patterns of community assembly in the developing chicken microbiome reveal rapid primary succession. Microbiologyopen. 2019;8.

38. Gong Y, Yang H, Wang X, Xia W, Lv W, Xiao Y, et al. Early intervention with cecal fermentation broth regulates the colonization and development of gut microbiota in broiler chickens. Front Microbiol. 2019;10 JUN.

39. Feng Y, Zhang M, Liu Y, Yang X, Wei F, Jin X, et al. Quantitative microbiome profiling reveals the developmental trajectory of the chicken gut microbiota and its connection to host metabolism. iMeta. 2023. https://doi.org/10.1002/imt2.105.

40. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One. 2019;14.

41. Zenner C, Hitch TCA, Riedel T, Wortmann E, Tiede S, Buhl EM, et al. Early-Life Immune System Maturation in Chickens Using a Synthetic Community of Cultured Gut Bacteria. mSystems. 2021;6.

42. Marcolla CS, Ju T, Willing BP. Cecal Microbiota Development and Physiological Responses of Broilers Following Early Life Microbial Inoculation Using Different Delivery Methods and Microbial Sources. Appl Environ Microbiol. 2023;89.

43. Ramírez GA, Richardson E, Clark J, Keshri J, Drechsler Y, Berrang ME, et al. Broiler chickens and early life programming: Microbiome transplant-induced cecal community dynamics and phenotypic effects. PLoS One. 2020;15 11 November.

44. Seidlerova Z, Kubasova T, Faldynova M, Crhanova M, Karasova D, Babak V, et al. Environmental impact on differential composition of gut microbiota in indoor chickens in commercial production and outdoor, backyard chickens. Microorganisms. 2020;8.

45. Siegerstetter SC, Schmitz-Esser S, Magowan E, Wetzels SU, Zebeli Q, Lawlor PG, et al. Intestinal microbiota profiles associated with low and high residual feed intake in chickens across two geographical locations. PLoS One. 2017;12.

46. Xi Y, Shuling N, Kunyuan T, Qiuyang Z, Hewen D, ChenCheng G, et al. Characteristics of the intestinal flora of specific pathogen free chickens with age. Microb Pathog. 2019;132:325–34.

47. McBride G, Parer IP, Foenander F. The Social Organization and Behaviour of the Feral Domestic Fowl. Animal Behaviour Monographs. 1969;2:125–81.

48. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol. 2009;7:887–94.

49. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. Nature. 2017;548:43–51.

50. Marcolla CS, Alvarado CS, Willing BP. Early life microbial exposure shapes subsequent animal health. Canadian Journal of Animal Science. 2019;99:661–77.

51. Impey CS, Mead GC, George SM. Competitive exclusion of salmonellas from the chick caecum using a defined mixture of bacterial isolates from the caecal microflora of an adult bird. Journal of Hygiene. 1982;89:479–90.

52. Stavric S, Gleeson TM, Blanchfield B, Pivnick H. Competitive Exclusion of *Salmonella* from Newly Hatched Chicks by Mixtures of Pure Bacterial Cultures Isolated from Fecal and Cecal Contents of Adult Birds. J Food Prot. 1985;48:778–83.

53. Gleeson TM, Stavric S, Blanchfield B. Protection of Chicks against *Salmonella* Infection with a Mixture of Pure Cultures of Intestinal Bacteria. Avian Dis. 1989;33:636.

54. Corrier DE, Hinton A, Hargis B, Deloach JR. Effect of Used Litter from Floor Pens of Adult Broilers on Salmonella Colonization of Broiler.

55. Corrier DE, Nisbet DJ, Scanlan CM, Hollister AG, Deloach JR. Environment and Health Control of Salmonella typhimurium Colonization in Broiler Chicks with a Continuous-Flow Characterized Mixed Culture of Cecal Bacteria 1.

56. Corrier DE, Nisbet DJ, Hollister AG, Scanlan CM, Hargis BM, Deloach JR. Development of Defined Cultures of Indigenous Cecal Bacteria to Control Salmonellosis in Broiler Chicks 1.

57. Corrier DE, David, Nisbet J, Ii JAB, Hargis BM, Keith NK, et al. Dosage Titration of a Characterized Competitive Exclusion Culture To Inhibit *Salmonella* Colonization in Broiler Chickens during Growout t. 1998.

58. Corrier DE, Nisbet DJ, Scanlan CM, Hollister AG, Caldwell DJ, Thomas LA, et al. Treatment of Commercial Broiler Chickens with a Characterized Culture of Cecal Bacteria to Reduce Salmonellae Colonization 1. 1995.

59. Schneitz C. Competitive exclusion in poultry - 30 years of research. Food Control. 2005;16 8 SPEC. ISS.:657–67.

60. Stern NJ, Cox NA, Bailey JS, Berrang ME, Musgrove MT. Comparison of Mucosal Competitive Exclusion and Competitive Exclusion Treatment to Reduce Salmonella and Campylobacter spp. Colonization in Broiler Chickens.

61. Ferreira AJP, Ferreira CSA, Knobl T, Moreno AM, Bacarro MR, Chen M, et al. Comparison of Three Commercial Competitive-Exclusion Products for Controlling Salmonella Colonization of Broilers in Brazil. 2003.

62. Revolledo L, Ferreira CSA, Ferreira AJP. Prevention of Salmonella Typhimurium colonization and organ invasion by combination treatment in broiler chicks. Poult Sci. 2009;88:734–43.

63. Zaytsoff SJM, Montina T, Boras VF, Brassard J, Moote PE, Uwiera RRE, et al. Microbiota Transplantation in Day-Old Broiler Chickens Ameliorates Necrotic Enteritis via Modulation of the Intestinal Microbiota and Host Immune Responses. Pathogens. 2022;11.

64. Wongkuna S, Kumar R, Ghimire S, Mattiello Drescher S. Identification of a microbial sub-community from the feral chicken gut that reduces Salmonella colonization and improves gut health in a gnotobiotic chicken model. bIOrXiv. 2022.

65. Nurmi E, Nuotio L, Schneitz C. The competitive exclusion concept: development and future. Int J Food Microbiol. 1992;15:237–40.

66. Vonaesch P, Anderson M, Sansonetti PJ. Pathogens, microbiome and the host: Emergence of the ecological Koch's postulates. FEMS Microbiology Reviews. 2018;42:273–92.

67. Sun Y, O'Riordan MXD. Regulation of bacterial pathogenesis by intestinal short-chain fatty acids. In: Advances in Applied Microbiology. Academic Press Inc.; 2013. p. 93–118.

68. Lev M, Forbes M. Growth response to dietary penicillin of germ-free chicks and of chicks with a defined intestinal flora. British Journal of Nutrition. 1959;13:78–84.

69. Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology. 2012;3 DEC.

70. Redweik GAJ, Millman ST, Parsons RL, Hurtado Terminel AN, Radkowski R, Daniels K, et al. Exposure to a Virtual Environment Induces Biological and Microbiota Changes in Onset-of-Lay Hens. Front Virtual Real. 2022;3.

71. Biagi E, Mengucci C, Barone M, Picone G, Lucchi A, Celi P, et al. Effects of Vitamin B2 Supplementation in Broilers Microbiota and Metabolome. Microorganisms. 2020;8:1134.

72. Teng P-Y, Kim WK. Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. Front Vet Sci. 2018;5.

73. Shanmugasundaram R, Lourenco J, Hakeem W Al, Dycus MM, Applegate TJ. Subclinical doses of dietary fumonisins and deoxynivalenol cause cecal microbiota dysbiosis in broiler chickens challenged with Clostridium perfringens. Front Microbiol. 2023;14.

74. Robinson K, Assumpcao ALF V., Arsi K, Erf GF, Donoghue A, Jesudhasan PRR. Effect of Salmonella Typhimurium Colonization on Microbiota Maturation and Blood Leukocyte Populations in Broiler Chickens. Animals. 2022;12:2867.

75. Greene G, Koolman L, Whyte P, Burgess C, Bolton D. The Gut Microbiota of Broilers Reared with and without Antibiotic Treatment. Microorganisms. 2023;11:876.

76. Ringseis R, Eder K. Heat stress in pigs and broilers: role of gut dysbiosis in the impairment of the gut-liver axis and restoration of these effects by probiotics, prebiotics and synbiotics. J Anim Sci Biotechnol. 2022;13:126.

77. Das Q, Shay J, Gauthier M, Yin X, Hasted T-L, Ross K, et al. Effects of Vaccination Against Coccidiosis on Gut Microbiota and Immunity in Broiler Fed Bacitracin and Berry Pomace. Front Immunol. 2021;12.

78. Varriale L, Coretti L, Dipineto L, Green BD, Pace A, Lembo F, et al. An Outdoor Access Period Improves Chicken Cecal Microbiota and Potentially Increases Micronutrient Biosynthesis. Front Vet Sci. 2022;9.

79. Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends in Biotechnology. 2015;33:496–503.

80. Degruttola AK, Low D, Mizoguchi A, Mizoguchi E. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis. 2016;22:1137–50.

81. Humphreys C. Intestinal Permeability.

82. Moya A, Ferrer M. Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance. Trends in Microbiology. 2016;24:402–13.

83. Barnes EM, Carter EL, Lewis JD. Predicting Microbiome Function Across Space Is Confounded by Strain-Level Differences and Functional Redundancy Across Taxa. Front Microbiol. 2020;11.

84. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006;124:837–48.

85. Dallas JW, Warne RW. Captivity and Animal Microbiomes: Potential Roles of Microbiota for Influencing Animal Conservation. Microbial Ecology. 2022.

86. Wienemann T, Schmitt-Wagner D, Meuser K, Segelbacher G, Schink B, Brune A, et al. The bacterial microbiota in the ceca of Capercaillie (Tetrao urogallus) differs between wild and captive birds. Syst Appl Microbiol. 2011;34:542–51.

87. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on humang-microbe mutualism and disease. Nature. 2007;449:811–8.

88. Duar RM, Lin XB, Zheng J, Martino ME, Grenier T, Pérez-Muñoz ME, et al. Lifestyles in transition: evolution and natural history of the genus Lactobacillus. FEMS Microbiol Rev. 2017;41 Supp_1:S27–48.

89. Frese SA, MacKenzie DA, Peterson DA, Schmaltz R, Fangman T, Zhou Y, et al. Molecular Characterization of Host-Specific Biofilm Formation in a Vertebrate Gut Symbiont. PLoS Genet. 2013;9:e1004057.

90. Hooper L V., Xu J, Falk PG, Midtvedt T, Gordon JI. A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proceedings of the National Academy of Sciences. 1999;96:9833–8.

91. Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature. 2010;464:908–12.

92. García-Amado M, Michelangeli F, Gueneau P, Perez M, Domínguez-Bello M. Bacterial detoxification of saponins in the crop of the avian foregut fermenter <i>Opisthocomus hoazin</i> J Anim Feed Sci. 2007;16 Suppl. 2:82–5.

93. Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? Trends in Ecology and Evolution. 2016;31:689–99.

94. Hird SM. Evolutionary Biology Needs Wild Microbiomes. Front Microbiol. 2017;8.

95. Henry LP, Bruijning M, Forsberg SKG, Ayroles JF. The microbiome extends host evolutionary potential. Nat Commun. 2021;12:5141.

96. Oh S, Lillehoj HS, Lee Y, Bravo D, Lillehoj EP. Dietary Antibiotic Growth Promoters Down-Regulate Intestinal Inflammatory Cytokine Expression in Chickens Challenged With LPS or Co-infected With Eimeria maxima and Clostridium perfringens. Front Vet Sci. 2019;6.

97. Lin J. Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. Frontiers in Microbiology. 2014;5 FEB.

98. Dibner JJ, Richards JD. Antibiotic Growth Promoters in Agriculture: History and Mode of Action.

99. Roura E, Homedes J, Klasing KC. Prevention of Immunologic Stress Contributes to the Growth-Permitting Ability of Dietary Antibiotics in Chicks ,. J Nutr. 1992;122:2383–90.
100. Taheri HR, Moravej H, Tabandeh F, Zaghari M, Shivazad M. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. Poult Sci. 2009;88:1586–93.
101. Krysiak K, Konkol D, Korczyński M. Overview of the Use of Probiotics in Poultry Production. Animals. 2021;11:1620.
102. Jha R, Das R, Oak S, Mishra P. Probiotics (Direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Animals. 2020;10:1–19.
103. Kubasova T, Seidlerova Z, Rychlik I. Ecological adaptations of gut microbiota members and their consequences for use as a new generation of probiotics. International Journal of Molecular Sciences. 2021;22.

CHAPTER 2: EARLY LIFE MICROBIAL EXPOSURE SHAPES SUBSEQUENT ANIMAL HEALTH

2.1. INTRODUCTION

The modern livestock industry is designed to optimize animal efficiency in order to meet the growing global demand for food products that are affordable and safe for consumption. During intensification of farming, dairy cows, pigs and chickens were moved from the outdoors into environmental controlled indoor barns. Enclosure protects livestock from predators, facilitates farming practices, and promote production efficiency; however, the high animal density can lead to infectious diseases outbreaks (Gilbert et al. 2017). To prevent pathogen infections, intensive livestock systems rely on strict biosecurity procedures, the use of vaccinations and antibiotics, and the physical separation of mature and young animals. Thus, farm animals are raised in conditions that are substantially different from the ones found in the natural world.

Microbial colonization in the gut starts at early stages of life, and the establishment of a complex and stable community is essential for the host's optimal growth and development at later stages (Bërgstrom et al 2014. Tamburini et al. 2016). At early life, manipulation of gut microbiota induced by environmental factors impact the expression of genes related with metabolism and compromise the energy homeostasis of the newborn. Modifications in host's metabolism increase the risk to develop metabolic disorders, such as obesity. Initial microbial colonization also modulates innate and adaptive immune responses, thus influencing host ability to mount adequate inflammatory responses and to resist disease.

The impact of the microbiota in host physiology is well recognized in the livestock industry, and, throughout the years, several methods have been developed to promote a "healthy microbiota" in farm animals; even though a proper definition of a "healthy microbiota" is still

lacking. In an ecological perspective, animals that harbour a beneficial microbiota may be better apt to survive and reproduce; and early microbial colonizers are more likely to thrive within the gut. It is hypothesized that a healthy microbiota resembles the microbiota that is transferred from healthy parents to their offspring; however, in modern rearing practices, this transfer is substantially minimized. Studies in humans pointed that modern lifestyle, including changes in diet, antimicrobial use and sanitation, lead to a phenomenon of a "disappearing microbiota", that has been linked to several metabolic and immune mediated diseases (Blaser, 2017). In comparison to their wild counterparts, domestic animals also have been submitted to dramatic changes in lifestyle. This review aims to convey information on how modern production practices influence microbial composition and the effects of microbial disruptions on animal metabolism, immune physiology, and disease resistance.

2.1.2 Gut health

Monitoring and maintaining animal health is fundamental for efficient livestock production. In particular, production efficiency is dependent upon optimal gastrointestinal health. In addition to its function in digestion and nutrient absorption, the gastrointestinal tract plays a pivotal role in immune development and regulation. It acts as a selective barrier, preventing the establishment of pathogenic microorganisms and their translocation towards the systemic system; while allowing the colonization by commensal microorganisms (Korver, 2006). Interestingly, the gut is the major site for pathogen exposure, and one of the most active immunological organs (Kogut and Arsenaut, 2017), thus requiring a delicate equilibrium: a certain level of immune stimulation is necessary to provide defense against threats. However, excessive stimulation can

hinder animal efficiency, as nutrients would be directed towards inflammatory and immune responses, and away from production (Colditz, 2002).

A healthy gut presents a certain level of resistance to microbial colonization in determined microenvironments, while supporting the colonization of others. The mucus layer contains mucin, a heavy glycosylated protein, produced by goblet cells, that function as a physical barrier against bacterial translocation. The mucus also contains lysozymes, secreted by Paneth cells, that can digest peptidoglycan from bacterial cell walls; lactoferrin, a protein that binds iron, reducing it availability as a substrate for microbial growth; and secretory IgA molecules that can bind to microbe-specific epitopes, preventing microbes from reaching the mucosal surface. Enterocytes, Paneth cells, and immune cells secrete antimicrobial peptides that can directly kill bacteria, and/or stimulate the recruitment of immune cells (Abreu, 2010). These barriers contribute to the compartmentalization of microbes within the lumen, limit microbial translocation towards the epithelial layer, and regulate the activation of immune responses (Hooper, 2009).

In homeostatic states, the gut is inhabited by a complex community of commensal microorganisms, that includes bacteria, archaea, eukaryotes, and virus. Microbial presence, cell components, and metabolites can influence host physiology, and have been shown to influence metabolic, infectious, and immune mediated diseases. The commensal microbiota helps to maintain a balance between unresponsiveness and initiation of immune responses (Sansonetti, 2004), and occupy available niches in the gut, acting as a barrier against potential pathogens (Costello et al. 2012). Commensal microbes are classified as autochthonous, or indigenous, when they stably colonize the gut, are present in most individuals, and offer significant contributions to the gut ecology; whereas allochthonous organisms (non-indigenous) are transient, and offer little contribution to gut dynamics (Savage, 1977).

Germ free animals have improper development of the immune system, including lower levels of secretory IgA in the intestine (Parry et al. 1977), fewer and smaller Peyer's patches, lower CD8+ and CD4+ T cells, lower production of antimicrobial peptides, lower expression of MHC class II molecules (Round and Mazmanian, 2009), and altered mucin composition (Forder et al. 2007). It was demonstrated that microbial colonization, particularly with a host-specific microbiota, is able to correct some of these defects (Mazmanian et al. 2005; Smith et al. 2007; Hrncir et al. 2008; Chung et al. 2012).

Microbial colonization tailors gastrointestinal development and modulates epithelial barrier function. Germ free animals have reduced enterocyte proliferation and turnover rate, lower TLR2 expression, and lower TNFα expression than conventional animals (Willing and Van Kessel, 2007). Although cell turnover has substantial metabolic costs, cell renewal is necessary to prevent accumulation of hazardous substances and to mend cellular lesions that could lead to barrier disruption (Kraehenbuhl et al. 1997). Toll-like receptors signaling have been demonstrated to have a role in sustaining homeostatic balance of intestinal tissues, reducing intestinal injury (Rakoff-Nahoum et al. 2004, Fagundes et al. 2012), and preserving tight-junction and barrier functions during inflammatory challenges (Cario et al. 2007).

In conventional animals, intestinal injuries can be followed by intense inflammatory responses and a high lethality rate. Conversely, in germ free animals, sterile intestinal injuries present low lethality, due to an inflammatory hyporesponsiveness state, mainly mediated by increased IL-10 production (Souza et al. 2004). However, when germ free animals happen to be contaminated with a pathogen, survival rates are significantly lower than those observed in conventional animals, as hindering of proinflammatory responses favours pathogen growth and dissemination (Fagundes et al. 2012).

The composition of the microbiota determines host-susceptibility to pathogens, and controls pathogen load in the intestinal environment. For example, transplantation of microbiotas from *Citrobacter rodentium*-resistant mice to susceptible mice resulted in reduced pathogen shedding and colonization, and delayed mortality, after a *C. rodentium* challenge (Willing et al. 2011). These effects were associated with increased expression of IL-22, a cytokine produced by leukocytes, that has been shown to increase expression of antimicrobial peptides (Abbas et al. 2008, Zheng et al. 2008). Besides regulating host immunity, commensal microbes can directly produce substances that inhibit pathogenic growth, such as bacteriocins and organic acids. In vitro studies demonstrated that some *E. coli* strains can produce antimicrobial proteins that inhibit enterotoxigenic *E. coli* growth (Schamberger and Diez-Gonzalez, 2002); butyric acid can downregulate the expression of virulence genes in *Salmonella* (Gantois et al. 2006); and acetic, propionic and butyric acid were shown to reduce growth of *E. coli* O157:H7 (Shin et al. 2002).

Animals raised in sanitized environments may lack a proper microbial colonization which modulates inflammatory responses and promotes host survival. This is similar to the observed in humans, in which changes in diet, behaviour, sanitation, and antimicrobial treatments have been linked to a loss of host-adapted microorganisms, and to an increase in the prevalence of chronic and immune-mediated diseases (Blaser and Falkow, 2009; Blaser, 2017). Germ-free mice colonized with cecal contents obtained from wild mice and challenged with Influenza virus had lower pro-inflammatory responses, and higher survival rate, than germ-free mice colonized with cecal contents obtained from laboratory mice (Rosshard et al. 2017). In a colorectal tumorigenesis model, mice colonized with wild-mice microbiota had lower weight loss and lower tumor development than mice colonized with lab-mice microbiota (Rosshard et al. 2017). These findings

support the concept that exposure and colonization by a host-adapted, naturally-selected microbiota may promote host health and survival.

In an ecological perspective, commensal microbes evolve to persist within the host; and hosts that harbour a predominantly beneficial microbiota may have higher chances to survive, and to transmit the beneficial microbiota to the next generation (Blaser and Falkow, 2009; Foster et al. 2017). To achieve a mutually beneficial relationship, commensal microbes need to thrive without triggering host immune responses that would result in their elimination, and metabolic burdens for the host (Foster et al. 2017). Thus, it is expected that the commensal organisms are mainly neutral, or beneficial (Figure 2.1). However, current production practices in the livestock industry, aiming at minimizing exposure to pathogens, may have inadvertently minimized the exposure of animals to their commensal microbes, and hindered the colonization of the gut by healthy, commensal, co-evolved microbes.

2.1.3. Concepts in Biosecurity

Biosecurity refers to standardized procedures that intend to protect humans, animals, and the environment against diseases and/or harmful biological agents. The World Organisation for Animal Health provides standards and guidelines for implementation of biosecurity procedures to be adopted in livestock production; also, countries may develop their own, based on risk assessment analysis, disease prevalence, and international trade requirements. The aim is to reduce the risk of occurrence of diseases that could affect animals and/or humans, cause declines in productivity, and negatively impact animal welfare and consumer perceptions of animal-based food products (CFIA, 2013).

A current management practice, especially in intensive systems, is to separate offspring from their parents, as early as possible, and house same age animals together, thus avoiding the contact between young and mature animals. In swine production systems, piglets are weaned at 3 or 4 weeks, moved to nursery facilities, grouped with same age piglets from different litters, and fed diets that are mostly plant-based (Johnson et al. 2001). In contrast, in nature, piglets start to occasionally consume solid feed between 3 and 4 weeks of age, and will only substantially increase the proportion of ingested solids after 5 weeks. Additionally, milk consumption does not cease before, at least, 8 weeks of age (D'Eath and Turner, 2009). In modern rearing, the last time piglets get directly in contact with a sow's mature microbiome is at 3 weeks old; whereas, in natural conditions, suckling behavior can still be observed in pigs that are older than 12 weeks (Jensen and Recén, 1989). Similarly, dairy calves are removed from cows at birth, housed individually and fed milk substitutes for 8 weeks, then group-housed with individuals of the same age; whereas, in nature, calves are progressively weaned throughout 10-months. Consequently, the exposure of dairy calves to microbes from mature animals is limited to the exposure they get from travelling the birth canal and being exposed to parental microbes for few hours after birth. The most extreme example comes from poultry production systems. In intensive farming, laid eggs are collected from nests as soon as possible, transferred to hatchery facilities, disinfected, and artificially incubated. After hatch, chicks are transported to empty and sanitized barns, and will never get in contact with the microbiome from mature birds, unless housed in barns that re-use bedding material from previous flocks (a practice not used in Canada).

Additional management practices in place include strictly cleaning and disinfection of facilities, depopulation between flocks/lots, and the use of prophylactic and growth promoter antibiotics. Although these procedures aim at reducing the contact between animals and potential

pathogens, and/or reduce pathogen load, they also inadvertently minimize the opportunities for the animals to be colonized with commensal organisms.

Despite the intensification of biosecurity measures, livestock animals are still major reservoirs of food borne pathogens and antibiotic-resistant organisms. In the United States alone, *Salmonella* infections are estimated to cause, annually, 1.2 million human illnesses and 450 deaths. The most common source of infection are animal-based food products, especially poultry meat and eggs, that have been contaminated with animal feces (CDC, 2018). Decades ago, Nurmi and Rantala (1973) demonstrated that 1-day-old chicks that received cecal content from mature chickens, via oral gavage, had lower *Salmonella* counts, compared to chicks that were not exposed to a mature microbiota. This finding highlighted the importance of microbial colonization, and the potential consequences of abnormal hygienic conditions in hindering the establishment of a commensal microbiota; and similar results have been consistently found in subsequent studies (Corrier et al. 1992; Andreatti Filho et al. 2003).

It is possible that livestock industry management practices to avoid contact between animals and pathogens have resulted, throughout the years, in a depletion of microbial species that co-evolved with these hosts. Some strategies to promote microbial colonization, such as feed additives containing prebiotics, probiotics, and yeast cell walls, have been implemented in the industry. However, beneficial effects of these products are inconsistently observed, and, when present, the effects usually cease when products are withdrawn from feed (Chichlowski et al. 2007; Frese et al. 2012). Thus, it is necessary to develop strategies to consistently and efficiently manipulate the gut microbiota towards the establishment of a host-adapted, stable and diverse microbiota, that can promote animal health and performance.

In livestock industry, although the beneficial aspects of the microbiota have been recognized for decades, precise manipulation of microbiota composition and identification of beneficial commensal species have been challenged by the inability to proper characterize microbial communities due to the reliance on culture-dependent techniques. However, recent advances in culture-independent methods and high-throughput sequencing technologies facilitated the study of bacterial taxonomy and functional characteristics, resulting in a better understanding of host-microbial interactions (Cho and Blaser, 2012), and highlighting the importance of co-evolved organisms, and specific microbial species on host physiology and disease resistance.

2.1.4. Host-adapted Microbiota

Even though individuals of the same species present substantial variation in their microbiota composition, the microbiota of members of the same species will frequently be more similar to one another than to those associated with individuals from another species (Ley et al. 2008). Interspecies differences are influenced by co-evolutionary processes, the availability of nutrients, and the environmental conditions within the host gut; whereas interindividual variations are mostly influenced by colonization early in life, diet, environmental exposures, and antibiotic use (Matamoros et al. 2013). Microbes have a rapid generation time, and microbial communities can change quickly in response to environment perturbations, thus, microbial shifts can facilitate host adaptation to new or changing environments (Alberdi et al. 2016).

Some studies suggested the existence of in utero (Jiménez et al. 2005; Ardissone et al. 2014) and in ovo (Pedroso et al. 2009; Ilina et al. 2016) microbiomes; however, these findings are arguable. Considering that germ-free status in animals derived via cesarean section, or sterilization of egg surface, is successfully obtained and sustained, studies pointing in that direction may be

resulting from contamination or inadequate experimental methodology (Perez-Muñoz et al. 2017). Therefore, it is assumed that animals are sterile within the uterus or the egg, and microbial colonization happens during the passage through the birth canal, or at hatch (Seed, 2015). In that way, early colonizers have greater chance to occupy and adapt to a niche, due to the lower level of competition (Costello et al. 2012). Additionally, due to their presence in fecal matter, it is expected that they will have greater likelihood of being transmitted between generations, as offspring get exposed to bacteria in the surrounding environment (Costello et al. 2012).

Colonization ability also depends on specific characteristics of bacterial species and strains (Lozupone et al. 2012), their adaptation to the host (Frese et al. 2012), and the existent microbiota (Maldonado-Gómez et al. 2016; Ju et al. 2017). Gene sequence databases, such as SILVA and Greengenes currently list more than one hundred reported bacterial phyla (Youssef et al. 2015); however, the majority of bacteria in the gastrointestinal tract of humans (Lozupone et al. 2012), pigs (Holman et al. 2017), and chickens (Kers et al. 2018; Yang et al. 2017) is restricted to relatively few phyla (Bacteroides, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia, Tenericutes, Fusobacteria), and largely dominated by Bacteroidetes and Firmicutes. This indicates that the gut is a selective environment, and supports the idea that co-evolutionary processes may benefit specific strains.

The presence of a bacterial species in the gut does not necessarily indicates its potential as a commensal. *Lactobacillus* strains used in probiotic products may not have the ability to colonize the mature gut, and may become undetectable in fecal samples after the consumption of probiotics ceases (Frese et al. 2012). Studies indicate that host-specific bacteria strains are more able to colonize compared to bacteria harvest from a different host-species. Duar et al. (2017) inoculated mice, chickens, pigs, and humans with different bacterial inoculums containing a mix of

Lactobacillus reuteri strains harvest from rodents, poultry, swine, and humans. After 5 days, chicken and mice showed significantly higher abundance of the host-specific strain, suggesting the existence of a host-adaptation advantage (Duar et al. 2017). Maldonado-Gómez (2016) administered *Bifidobacterium longum* to humans and found that a successful colonization correlates with resource availability and functional and taxonomic characteristics of the gut, such as low abundance of *B. longum* species, and diminished expression of genes involved in carbohydrate metabolism. Absence or low abundance of a host-specific microorganism seem to positively influence microbial ability to successfully colonize the gut after exposure. Mice lacking commensal *E. coli* and *Parasutterella* can be colonized with a single dose of these organisms, via gavage or by inoculating in bedding material, respectively; without causing significant changes in microbiota composition and biodiversity (Ju et al. 2017; Ju et al. 2019). Interestingly, without disturbing the existent microbial community, these organisms were shown to promote significant changes in host metabolism, such as altering bile acids profiles, and affecting host responses to antibiotics (Ju et al. 2017; Ju et al. 2019). However, due to colonization resistance, even autochthonous organisms may eventually fail to efficiently colonize and persist within the gut (Jacobsen et al. 1999; Frese et al. 2012).

2.2. MICROBIAL DISRUPTIONS

2.2.1. Antibiotics

Antibiotics are frequently employed in the livestock industry, to treat and prevent diseases, and to improve production efficiency. There is mounting pressure to reduce the use of these compounds, especially for growth promotion purposes; still, the annual consumption of antibiotics by farm animals is projected to rise, and to reach more than 105 tons by 2030 (Van Boeckel et al.

2015). Besides the risk of increasing antimicrobial resistance, the use of antibiotics can promote changes in animal microbiota and immune development. The magnitude and duration of these effects are yet to be defined. Although numerous studies have been conducted to evaluate antibiotics impact on livestock microbiota, the existent findings are hard to integrate. Due to differences on antimicrobial compounds and doses used, age of administration, animal models, and analysis performed, the results across studies are frequently inconsistent.

2.2.1.1. Antibiotic-driven immune disruptions

Antibiotics stimulate changes in microbiota dynamics and composition, and can directly and indirectly influence host immune response and disease-resistance (Willing et al. 2011b). The consequences of antimicrobial use are particularly relevant in early life. Studies in humans have shown correlation between antimicrobial treatment during infancy, and the occurrence of immune mediated diseases, such as asthma (Ahmadizar et al. 2017), atopy (Fujimura et al. 2015), and inflammatory bowel diseases (Hviid et al. 2011). Antibiotics can reduce community diversity, select for resistant bacterial strains, and deplete commensal populations, thus favoring the growth of opportunistic pathogens (Willing et al. 2011b). Antibiotics were shown to reduce the production of pro-inflammatory cytokines, antimicrobial compounds (Menendez et al. 2013), and mucin (Wlodarska, et al. 2011). Animals treated with antibiotics may present loss of pattern recognition receptors function, reduced serum immunoglobulin levels, and downregulation of antigen presenting genes (reviewed in Willing et al. 2011b). These changes in microbiota dynamics and host physiology can affect gastrointestinal integrity, and the animal ability to overcome disease challenges.

Even when changes in microbiota composition seem to be transient, antibiotic treatment may have long-lasting effects on host physiology. Administration of 0.8 mg amoxicillin to 1-day-old broilers caused discrete changes in microbial composition evaluated at 5 days old, which were no longer observed at 14-days-old. However, at 14-days-old, antibiotic-treated birds showed lower numbers of macrophage-like cells in the jejunum, compared to control birds (Schokker et al. 2017). In some cases, changes caused by antibiotic treatment may be restored by microbiota manipulation. Broiler chickens treated with antibiotics for one week after hatch showed reduced numbers of regulatory T cells and lower expression of IL-10 and IFNγ in cecal tonsils. However, after co-housing with untreated chickens, regulatory T cell numbers in antibiotic-treated chickens increased, and significant differences between the two groups were no longer observed one week after co-housing (Lee et al. 2018). In the same experiment, acetate administration restored regulatory T cell population in antibiotic-treated chickens, further indicating that antibiotics effects on host immune cells resulted from disruption of the microbial community (Lee et al. 2018).

In piglets, antibiotic treatments have shown diverse effects on microbial composition and immune modulation. Amoxicillin administration (15mg/kg) to 1-day-old resulted in decreased microbial diversity, and increased abundance of enterobacteria, such as *Shigella spp*. *E. coli* and *Salmonella enterica* serovar Typhi, in cecal samples collected 40 days after treatment (Janzcyk et al. 2007). Administration of tulathromycin (2.5mg/kg) to 4-day-old piglets increased microbial diversity, and the abundance of anaerobic bacteria, such as *Bifidobacterium*, *Eubacterium*, *F. prausnitzii,* whereas decreased facultative anaerobes such as *S. aureus*, in digesta samples collected 4 days after treatment (Schokker et al. 2014). Piglets raised in micro isolators and treated with antibiotics for 56 days after birth showed higher abundance of Proteobacteria, mainly Enterobacteriaceae, and lower expression of TLR2, PM22, and other genes involved in

inflammatory responses, T cell signaling, and leukocyte-endothelial cell interactions, compared to piglets raised outdoors (Mulder et al. 2009). Toll-like receptor 2 and peripheral myelin protein 22 (PM22) are regulators of tight junction proteins function (Notterpek et al. 2001, Cario et al. 2007), and their downregulation is associated to impairment of gut barrier integrity (Cario et al. 2007; Wang et al. 2015). Expression of TLR-2 has also been linked to a better immune response in piglets vaccinated against porcine circovirus-2 (Chen et al. 2018).

2.2.1.2. Antibiotic-driven metabolic disruptions

In addition to impacts on immune development and function, disruptions in the early life microbiome have been shown to impact subsequent metabolic health. Perturbations in the gut microbiota establishment during the first weeks to months of life caused by environmental factors such as antibiotics exposure, changes in diet composition, (Wang et al 2017), including the sow´s diet, and stress during weaning have an impact on bacterial diversity and abundance of selected taxa, resulting in changes in microbial metabolic function (Tanaka et al. 2017). Microbes contribute to the production of in vitamins, amino acids, bile acids and short-chain fatty acids (SCFA), which the later provide mechanisms through which they are involved in regulation of glucose and lipid metabolism, gut motility and energy storage (Rowland et al. 2018).

From birth, animals can be exposed to therapeutic and subtherapeutic doses of antibiotics for different purposes, resulting in varying doses and antimicrobial spectra. It is hypothesized that early life is a critical window where shifts in gut microbiota community induced by antibiotic exposure can affect the stability and diversity of bacteria composition and host's gene pathways related with metabolism and immune system (Bokulich et al. 2017) The etiology of metabolic disorders and autoimmune disease remains unclear; however, an increase evidence describe the

molecular mechanism driven by gut microbiota that regulate metabolism and immune activity, highlighting a possible crosstalk of gut microbiota with different organs (Cox et al 2014, Schokker et al. 2014).

Cox et al. was one of the first groups to demonstrate a causal link between early life antibiotic exposure and altered metabolic outcomes. They demonstrated that administration of subtherapeutic doses of penicillin at early stages of life in mice caused a shift in *Lactobacillus, Candidatus Arthromitus* and *Allobaculum.* Despite the recovery of the microbiome, mice exposed to antibiotic had an increased expression of genes related to hepatic lipid metabolism (*Pparγ, Cd36* and fatty acid binding protein 2 (Fabp2)) and developed significantly higher fat-mass compared to control group. The introduction of a high-fat diet accelerated the development of obesity at 16-20 weeks of life in mice treated with antibiotic in early life (Cox et al. 2014).

Increased circulating levels of leptin and gastric inhibitory polypeptide (GIP) in piglets and mice have been reported as short-term effects of antibiotics use during the first days of life (Cho et al 2012. Ipharraguerre et al 2018). GIP is an incretin secreted by K enteroendocrine cells that induce the secretion of insulin from pancreatic islets. The deficiency or impairment of GIP secretion is a factor that contributes to β-cell failure, a common characteristic of type 2 diabetes pathophysiology (DeFronzo R. et al. 2015). Leptin is a hormone secreted by the adipose tissue that modulate energy metabolism by promoting insulin sensitivity in liver, muscle and adipose tissue. In obesity, elevated concentration of leptin is associated with the up regulation of TNFa and IL-6, proinflammatory cytokines that reduce insulin responses and β-cell failure (López-Jaramillo et al. 2014). Therefore, the changes identified in metabolic molecules at early life could explain the development of obesity and type-2 diabetes phenotype seen in adulthood.

The obese-phenotype development as a long-term effect of early-life antibiotic exposure is associated with the alteration of SCFA metabolism. SCFA provide 60-70% of energy to colonocytes and are endogenous ligands of different G-protein coupled-receptors involved in glucagon and insulin secretion. In addition, SCFA play an important role in enterocyte proliferation and adipocyte differentiation through the activation of peroxisome proliferator-activated receptor-gama (PPARγ) (Li et al. 2017). Piglets exposed to antibiotic cocktail (ampicillin, metronidazole and gentamycin) during the first days of life presented a decreased abundance of total bacteria Firmicutes, Bacteroidetes, *Lactobacillus, Bifidobacterium, Clostridium, Megasphaera,* and *Ruminococcus* in ileum and feces. The changes of these microbial populations positively correlated with the decrease in the concentration of acetate and butyrate (Gao et al. 2018, Pi et al. 2019). Acetate, butyrate and propionate are the main SCFA produced by microbial fermentation in the cecum. Butyrate induces the expression of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), which is a transcription factor that promotes lipid oxidation and glucose metabolism in adipose tissue and muscle (Baht et al. 2017). Cho et al. identified a decrease in gene expression related with butyrate synthesis in mice exposed to antibiotics during the first weeks of life. At later stages of life, the same group of mice presented an increase in adiposity compared to control group (Cho et al. 2012).

Li et al. demonstrated that exposure of antibiotic at early life induce changes in gene expression of SCFA receptors in the colon, but also changes in pancreatic cell function that was related with impaired glucose metabolism in the pancreas at later life. The piglets exposed to antibiotic presented increased insulin secretion, altered growth and apoptosis rate of b-cells, and changes in the expression of genes related with pancreatic cell growth and proliferation (PDX-1 and IGF2) (Li et al. 2017). The findings suggest that administration of antibiotic at early stages

can induce changes in metabolic organ performance and enhance the development of an obese or type 2 diabetes phenotype. It is necessary to study the mechanisms driving the crosstalk of microbiota with other metabolic organs such as the pancreas and explore the effects on the physiology of the host in health and disease.

Antibiotics also induce changes in bile acid metabolism by decreasing intestinal bacteria population with bile acid hydrolase activity (BSH), such as *Lactobacillus* and *Clostridia XIVa* and *Clostridia IX* (Ipharraguerre et al. 2018). Bile acids are endogenous ligands for nuclear receptor farnesoid X receptor (FXR) and the G-protein coupled receptor for bile acids (TGR5). FXR activation maintains the intestinal barrier and regulates de novo synthesis of cholesterol in the liver. TGR5 regulates energy homeostasis in adipose tissue and muscle, while its expression in the pancreas and intestine regulates insulin signalling (Joyce 2016). Ipharraguerre et al showed that amoxicillin and chlortetracycline induce changes in primary and secondary bile acid ratio. In addition, leptin and adiponectin serum concentration was increased in piglets treated with amoxicillin, but plasma insulin concentration was decreased in chlortetracycline treated group (Ipharraguerre et al. 2018). The findings of this study suggest that a reduction of bacteria affects production of metabolites that promote anabolic functions of the host, and those changes could have negative metabolic outcomes.

2.2.2. Domestication and intensive farming

Along the domestication process, wild animals underwent confinement, changes in diet, and artificial selection, resulting in the modern livestock animals that fulfil specific human needs. It is expected that these environmental, dietary, and genetic changes would impact microbiota composition, and thus influence animal health and disease resistance. Metagenomic analysis of

swine fecal samples revealed that samples obtained from wild and domesticated hogs kept in captivity, either in a zoo or in commercial farms, were phylogenetically closer, and clustered separately from samples from free range domesticated and wild hogs (Ushida et al. 2016). *Clostridium* had the highest relative abundance, in both domestic and wild pigs; and pigs kept in commercial farms had higher relative abundance of *Lactobacillus* and lower *Bifidobacterium* and *Ruminococcus*. Comparison of microbiota composition between wild and domestic turkeys, using oligonucleotide fingerprinting of ribossomal RNA gene analysis, revealed that they have similar levels of community richness and evenness, but share only about 37% of the operational taxonomic units (Scupham et al. 2008). Analysis of cecal content from free-range, feral chickens, and commercial broilers using UniFrac distance matrix indicated that samples from free-range and feral chickens clustered separately from broiler samples, even though free range and feral chicken samples were collected from different locations (Europe and Bermuda) (Ferrario et al. 2017). The same study showed that broiler microbiota lacks "core" species that are found in free-range and feral chickens.

Mulder et al. (2009) found that weaned piglets born from sows housed outdoors have lower microbial diversity than piglets born from indoor-raised sows. Also, in piglets obtained from outdoors the microbial community is dominated by Firmicutes, mostly Bacilli; whereas in indoor communities, Bacteroidetes, particularly the *Prevotellaceae* family, were more abundant. The abundance of Proteobacteria, mainly *Enterobacteriaceae*, was shown to be higher in piglets raised in cleaner environments, and to negatively correlates with Firmicutes abundance (Mulder et al. 2009). It would be expected that access to outdoors increase microbiota diversity, but this is not always observed. Studies comparing diversity in free range and confined livestock are scarce and show inconsistent results. Cui et al. (2017) found that free range chickens present higher microbial

diversity than caged hens; conversely, Sun et al. (2018) found higher diversity in caged hens. An investigation on the effects of captivity in woodrats has shown that captivity leads to a reduction on microbial diversity, that is only partially recovered when animals are fed natural diets (Kohl et al. 2014).

The increase in Proteobacteria observed in confined animals may reflect a less beneficial microbiota. During inflammatory responses, alteration of microbiota dynamics leads to reduction in obligate anaerobes, (Lupp et al. 2007), favoring the growth facultative anaerobes, particularly Proteobacteria, which have been linked to dysbiosis and intestinal inflammation (Bäumler and Sperandio, 2016). In the microbiome of confined animals, the reduction of beneficial members of the Firmicutes phylum, such as *Lactobacillus*, *Faecalibacterium*, and *Ruminococcus*, is seen as detrimental. However, genus information is often insufficient for interpretation. For example, some *Lactobacillus* (*L. crispatus*, *L. reuteri* and *L. vaginalis*) have shown positive correlation to chicken feed efficiency, whereas others have been linked to poor performance (*L. salivarius*, *L. agilis*, and L. *saerimneri*) (Crisol-Martínez, 2017). Interestingly, *Lactobacillus* isolates obtained from free-range chickens showed in vitro antagonistic activity against selected pathogens more frequently than isolates from commercial broilers (Souza et al. 2007). The inhibitory effects of *Lactobacillus* isolates from Guinea fowl, in combination with mannan oligosaccharides, have been shown to reduce intestinal loads of *Salmonella spp*. and *E. coli* in broilers (Vineetha et al. 2016). *Faecalibacterium prausnitzii*, a butyrate-producer that has been negatively associated with confinement, has been shown to affect host physiology in several ways: it decreases gene expression of pro-inflammatory mediators, including NO, IL-6 and IL-12 (Chang et al. 2013), improves epithelial barrier function (Zheng, 2017), and induces regulatory T-cells differentiation (Furusawa et al. 2013). It is a commensal bacterium in chickens, pigs, and calves (Miquel et al.

2014), and its oral administration to preweaned calves was shown to reduce the incidence of severe diarrhea and increase weight gain (Foditsch et al. 2015). Bearson et al. (2014) found that pigs that shed little *Salmonella* when challenged present higher *Ruminococcaceae* abundance before challenge compared to high shedders; indicating that this bacteria can potentially be important in preventing pathogen establishment in the gut.

Promoting the colonization of offspring with parental microbiota may be the first step to explore the potential of host-adapted bacteria. Piglets raised in micro isolators and inoculated with boar feces at 33-day-old showed increased microbial richness and diversity; higher abundance of Firmicutes, *Barnesiella*, *Roseburia*, and *Thermovirga*; and lower abundance of *Dorea* and *Blautia*, than piglets that were not inoculated. Also, inoculated piglets showed stronger cell mediated response after an *Ascaris suum* extract skin test; and lower lung lesions scores and delayed onset of coughing after a *Mycoplasma hyopneumoniae* challenge (Schachtschneider et al. 2013). Chickens raised on reused bedding material tended to have lower abundance of *C. perfringens* compared to chickens raised in fresh bedding (Wei et al. 2013). After a *Salmonella* Enteritidis challenge, chickens inoculated with bacteria cultured from cecal contents of mature birds show lower infection rate and shedding (Andreatti Filho et al. 2003). However, the outcomes of microbial exposure are still highly unpredictable, possible due to the variability of the microbiota of donors and recipients (Stanley et al. 2013; Kers et al. 2018). Donaldson et al. (2017) exposed the egg surface to cecal contents obtained from low or high efficient broilers, and, counter to hypothesis, growth efficiency was lower in broilers hatched from eggs inoculated with cecal content from efficient birds.

In addition, artificial selection for immune traits may play a role on microbial composition. Layer hens selected for high or low antibody response to SRBC, raised in similar conditions, show

similar microbial diversity, but distinct abundance of 21 genera. When genetic selection is relaxed, high antibody responders chickens showed increase in *Ruminococcaceae*, *Oscillospira*, and *Sutterella* abundances; whereas low antibody responders showed increases in *Lactobacillus*, *Pseudomonas* and *Oscillospira*. Unweighted Unifrac (taxonomic and presence/absence data) based analysis showed that microbial communities of the four lines clustered differently, thus indicating that host humoral immunity influences microbiota composition (Yang et al. 2017). Chickens selected for higher mannose-binding lectin plasma concentration exhibited lower *Salmonella infantis* shedding after an oral challenge, and higher daily body weight than chickens selected for low mannose-binding lectin plasma concentration (Ulrich-Lynge, 2015).

A highly diverse ecosystem is usually considered more stable and resilient (Bäcked et al. 2005), and decreased microbial taxonomic diversity has been frequently associated with inflammatory (Ott et al. 2004; Belkaid and Hand, 2014) and infectious diseases (Chang et al. 2008; Caballero and Pamer, 2015) in humans and mouse models. Stanley et al. (2016) found that the microbiota diversity is higher in highly efficient chickens. However, the functional diversity of the microbiota must also be taken into consideration. Studies in humans have demonstrated that, although the microbial community is highly different between subjects, the functional diversity is quite similar, indicating that core functions may be performed by different microbial species (Lozupone et al. 2012). By comparing the microbiomes of domestic animals and wild populations, it may be possible to identify microorganisms that were lost during domestication, and to develop strategies to re-introduce these beneficial commensals to livestock, aiming to obtain positive outcomes in performance and immune responses.

2.2.3. The impact of maternal diet on offspring microbiota

Maternal transmission of bacteria is one of the crucial factors influencing the establishment and shape of gut microbiota community in the offspring. Newborn piglet acquires maternal bacteria by the exposure to the sow's feces, skin and nipple surface, and by feeding on milk (Chen et al. 2017).

Maternal diet composition during gestation and lactation impacts the newborn's health and growth, by altering the composition of the milk as well as impacting the microbes they transfer to the offspring. Piglets from sows fed with a high-fiber diet maintained weight gain (from day 1 to day 21) and showed an increased in acetate molar ratio (Guillemet et al. 2007; Chen et al. 2017). Moreover, the piglets showed higher plasma concentrations of insulin growth factor-1 (IGF-1), IL-10 and TGF-β, which are implicated in anti-inflammatory process, and a reduction of inflammatory factors such as fecal lipocalin-2, a marker for intestinal inflammation (Yan et al., 2015).

Conversely, high-fat diet has been associated with the disruption of metabolic functions of the gut microbiota and as consequence gives rise to the development of obesity. Yucatan minipigs sows feed with high-energy content diet (high fat diet or sucrose; 167% of the energetic level in comparison to control group) produced milk with higher lipid fraction, and presented an increased circulating levels of cholesterol and free-fatty acids as compared to control. Moreover, high fat/sucrose maternal diet had an impact on piglet's metabolic performance and gut microbiota function (Val-Laillet al. 2017). The fermentation of SCFA in piglets from mothers fed with high-fat and high sucrose diet was not different at the age of 14 and 39 day-old, but a decrease in propionate, butyrate and acetate concentration in fecal samples were detected at day 100. In addition, circulating levels of free-fatty acids and triglycerides were detected in the same piglets

(Val-Laillet et al. 2017). These evidence shows that maternal gut microbiota contribute to health state of the offspring and the transmission of an altered bacteria population could predispose the offspring to develop an obese-phenotype.

2.2.4. Weaning

As mentioned, intensive rearing practices include the early and abrupt separation between parents and offspring, which imposes significant challenges to the young. Besides the high nutrient content and high digestibility, maternal milk contains oligosaccharides, hormones, immunoglobulins, and other bioactive compounds that stimulates infant growth, gastrointestinal and immune development, and inhibit colonization by pathogens (Odle et al. 1996; Martin et al. 2016). Milk contain oligosaccharides and glycoproteins that can bind to pathogenic bacteria receptors, thus preventing adhesion to host cells (Newburg, 2000). The substrates present in milk also play a role in selecting bacteria that can utilize oligosaccharides and lactose, such as *Bifidobacteria* and *Lactobacillus* (Matamoros, 2013), that are considered to have health-promoting effects and are frequently included as probiotic in feed (Gomes and Malcata, 1999). These species can ferment carbohydrates and produce acetic and lactic acid, which causes decreases in pH that can disfavor pathogenic growth (Castellano et al. 2017). Administration *Bifidobacteria* and *Lactobacillus* species to piglets and calves was shown to promote body weight gain, improve feed conversion, and reduce diarrhea and mortality occurrence (Abe et al. 1995). In humans, it was demonstrated that breastfed infants have higher abundance of *Bifidobacterium*, *Bacteroides*, and lactic acid bacteria, including *Lacobacillus*; whereas formula-fed infants have higher abundance of *Staphylococci* and *Clostridia* (Harmsen et al. 2000). Also, it is suggested that premature

weaning is linked to diarrhea, infectious diseases, and autoimmune disorders occurrence (Jackson and Nazar, 2006).

In swine production, weaned piglets are moved to nursery rooms, where they are mixed with piglets from different litters. This leads to hierarchical disputes and other undesired behaviours, such as cannibalism, vocalization and nosing, which negatively affect animal welfare (Colson et al. 2006). However, the major challenge for weaned piglets is to initiate the consumption of solid feed, that present lower digestibility and lack protective and immunomodulatory factors present in milk. This, associated with intestinal immaturity, can result in transient anorexia, villi atrophy, and crypt hypertrophy, consequently reducing nutrient digestion, absorption, and growth rate (De Passillé et al. 1989; Pluske et al. 1997; Mormède and Hay, 2003), and increasing intestinal permeability to antigens and toxins (Bomba et al. 2014). Nutrient malabsorption and the reduction on mature enterocytes numbers increase susceptibility to pathogen infections, specially to enterotoxigenic *Escherichia coli* species, resulting in dysbiosis and post weaning diarrhea (Konstantinov, 2006).

Weaning is reported to cause a loss of microbial diversity, including reduction on *Lactobacillus* species and increase in facultative anaerobes and potential pathogens, such as Proteobacteria, *Clostridium* spp, *Prevotella* spp. and *E. coli* (Konstantinov, 2006; Gresse et al. 2017). Some *Lactobacillus* species are reported to outcompete pathogens for nutrients and attachment sites, and to produce lactic acid and bacteriocins that can reduce pathogen growth (Hou et al. 2015), thus their reduction may promote pathogen proliferation after weaning. Weaning leads to gut inflammation and disruption of barrier function, reducing the expression of tight-junction proteins, such as occludin and zonula occludens (Wei et al. 2016). The increased concentration of reactive oxygen species and pro inflammatory cytokines, TNFα, IL-1β, and IL-6, favours the

expansion of facultative anaerobes, such as *Enterobacteriaceae* and reduces the abundance of commensal obligate anaerobe bacteria (Winter et al. 2013).

2.3. CONCLUSION

Modern rearing practices impact microbial community composition, leading to changes in microbial metabolites that affect host health and physiology. These changes in host responses can also influence microbial community dynamics (Figure 2.2). Culture-independent techniques have allowed the identification of microbial shifts that correlate to physiological outcomes; however, due to the complexity of the microbial-microbial and microbial-host interactions, it is still not possible to precisely determine which mechanisms lead to observed changes.

Findings of different studies identified the first days of life as a critical window for the establishment of a stable and diverse bacteria community that influence host genetic programming of the immune system and metabolism. The detection of differential expression of multiple genes involved in the production of inflammatory cytokines, glucose, lipids and bile acids metabolism in early life are used as reference to predict outcomes and phenotypes manifested in adulthood. Gut microbiota disruptions have a significant association with changes in the physiological function of organs involved in energy homeostasis and inflammatory status of production animals. Studying the molecular mechanism driven by gut microbiota in host gene programming during the first days of life could contribute to identification of pathways and key factors that impact host physiology.

Compared to the host, the gut microbiota has a greater potential to quickly adapt to changes in environmental conditions. Although the microbiota of domestic and wild animals present a certain level of similarity, the abundance of certain genera is substantially different between these

groups, and between domestic animals raised in intensive or extensive systems. Understanding how modern rearing practices impact microbial population is the first step to identify beneficial microorganisms that could potentially be introduced to promote positive physiological outcomes. Historically, it has been demonstrated that inoculation with fecal matter obtained from mature animals can promote immune maturation and improve disease resistance, particularly in chickens; however this practice is no longer acceptable due to the risk of introduction of pathogenic organisms, and diseases outbreaks.

By elucidating microbial shifts and the host patterns of response it may be possible to identify specific microorganisms that are responsible for substantial physiological changes. Culturing, isolation and introduction of these "major players" to domestic animals can be used as a strategy to promote proper microbial colonization, without increasing the risk of disease outbreaks. Examining the outcomes of gut microbiota manipulation can guide the development of novel strategies to prevent metabolic disorders, improve productivity, and modulate inflammatory responses. In the future, it may be possible to artificially select and promote colonization with specific microbes that positively impact production efficiency and animal health, in a way that resembles the selection for specific animal genetic traits that was successfully achieved in modern farming.

2.4. REFERENCES

Abbas, A.R., Modrusan, Z., Gong, Q., Zheng, Y., Sa, S.M., Ouyang, W., Danilenko, D.M., Valdez, P.A., Hu, Y., Ghilardi, N. and de Sauvage, F.J. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14: 282-289.

Abe, F., Ishibashi, N. and Shimamura, S. 1995. Effect of Administration of Bifidobacteria and Lactic Acid Bacteria to Newborn Calves and Piglets. J. Dairy Sci. 78:2838–2846.

Abreu, M.T. 2010. Toll-like receptor signaling in the intestinal epithelium: How bacterial recognition shapes intestinal function. Nat. Rev. Immunol. 10:131–143.

Ahmadizar, F., Vijverberg, S.J.H., Arets, H.G.M., de Boer, A., Turner, S., Devereux, G., Arabkhazaeli, A., Soares, P., Mukhopadhyay, S., Garssen, J., Palmer, C.N.A., de Jongste, J.C., Jaddoe, V.W.V., Duijts, L., van Meel, E.R., Kraneveld, A.D., and Maitland-van der Zee, A.H. 2017. Early life antibiotic use and the risk of asthma and asthma exacerbations in children. Pediatr. Allergy Immunol. 28:430–437.

Alberdi, A., Aizpurua, O., Bohmann, K., Zepeda-Mendoza, M.L. and Gilbert, M.T.P. 2016. Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? Trends Ecol. Evol. 31:689–699.

Andreatti Filho, R.L., Marcos Sampaio, H., Rodrigues Barros, M., Gratão, P.R, and Cataneo, A. 2003. Use of cecal microflora cultured under aerobic or anaerobic conditions in the control of experimental infection of chicks with Salmonella Enteritidis. Vet. Microbiol. 92:237–244.

Ardissone, A.N., De La Cruz, D.M., Davis-Richardson, A.G., Rechcigl, K.T., Li, N.J., Drew, C.R., Murgas-Torrazza, R., Sharma, M.L., Hudak, E.W., Triplett, and Neu. J., 2014. Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One. 9:1–8.

Bäumler, A.J. and Sperandio, V., 2016. Interactions between the microbiota and pathogenic bacteria in the gut. Nature. 535:85–93.

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A. and Gordon J.I., 2005. Host-Bacterial Mutualism in the Human Intestine. Science. 307:1915–1920.

Baht, M.I. and Kapila R. 2017. Dietary metabolites derived from gut microbiota:critical modulators of epigenetic changes in mammals. Nutr. Rev. 75(5):374-389.

Bearson, S.M.D., Allen, H.K., Bearson, B.L., Looft, T., Brunelle, B.W., Kich, J.D., Tuggle, C. K., Bayles, D.O., Alt, D., Levine, U.Y. and Stanton, T.B. 2013. Profiling the gastrointestinal microbiota in response to Salmonella: Low versus high Salmonella shedding in the natural porcine host. Infect. Genet. Evol. 16:330–340.

Belkaid, Y. and T. W. Hand. 2014. Role of the microbiota in immunity and inflammation. Cell. 157:121–141.

Bergström, A., Skov, T.H., Bahl, M.I., Roager, H.M., Christensen, L.B., Ejlerskov, K.T., Mølgaard, Michaelsen K.F,. Licth T.R. 2014. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of large cohort of Danish infants. App. Environ. Microbiol. 80(9): 2889-2900.

Blaser, M. J. 2017. The theory of disappearing microbiota and the epidemics of chronic diseases. Nat. Rev. Immunol. 17:461–463.

Blaser, M. J., and Falkow, S., 2009. What are the consequences of the disappearing human microbiota? Nat. Rev. Microbiol. 7:887–894.

Bokulich, N.A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H.D., Lieber, A., Wu, F., Perez-Perez, G.I., Chen, Y., Schweizer, Zheng, X., Contreras, M., Dominguez-Bello, M.G. and Blaser M.J. 2016. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 8(343): 1-25.

Bomba, L., Minuti, A., Moisá, S. J, Trevisi, E., Eufemi, E., Lizier, M., Chegdani, F. Lucchini, F., Rzepus, M., Prandini, A., Rossi, F., Mazza, R., Bertoni, R., Loor, J.J. and Ajmone-Marsan, P.

2014. Gut response induced by weaning in piglet features marked changes in immune and inflammatory response. Funct. Integr. Genomics. 14:657–671.

Caballero, S. and E. G. Pamer. 2015. Microbiota-Mediated Inflammation and Antimicrobial Defense in the Intestine. Ann. Rev. Imm. 33:227–256.

Cario, E., G. Gerken, and D. K. Podolsky. 2007. Toll-Like Receptor 2 Controls Mucosal Inflammation by Regulating Epithelial Barrier Function. Gastroenterology. 132:1359–1374.

Castellano, P., Pérez Ibarreche, M., Blanco Massani, M., Fontana, C. and Vignolo, G. 2017. Strategies for Pathogen Biocontrol Using Lactic Acid Bacteria and Their Metabolites: A Focus on Meat Ecosystems and Industrial Environments. Microorganisms. 5:38.

Centers for Disease Control and Prevention (CDC). Available at https://www.cdc.gov/salmonella/index.html

Chang, J. Y., Antonopoulos, D.A., Kalra, A., Tonelli, A., Khalife, W.T., Schmidt, T.M. and V.B. Young. 2008. Decreased Diversity of the Fecal Microbiome in Recurrent Clostridium difficile – Associated Diarrhea. J. Infect. Dis. 197:435–438.

Chang, P. V., Hao, L., Offermanns, S. and Medzhitov, R. 2014. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc. Natl. Acad. Sci. 111:2247–2252.

Chen, Y., Song, T., Le Xiao Y., Wan, X., Yang, L., Li, J., Zeng, G., Fang, P., Wang, Z.Z. and Gao, R. 2018. Enhancement of immune response of piglets to PCV-2 vaccine by porcine IL-2 and fusion IL-4/6 gene entrapped in chitosan nanoparticles. Res. Vet. Sci. 117:224–232.

Chen, X., Xu, J., Ren, E., Su, Y. and Zhu, W. 2018. Co-occurrence of early gut colonization in neonatal piglets with microbiota in the maternal and surrounding delivery environments. Anaerobe 49:30-40.

Chichlowski, M., Croom, J., McBride, B.W., Haveinstein, G.B., and Koci, M.D. 2007. Metabolic and Physiological Impact of Probiotics or Direct-Fed-Microbials on Poultry: a brief review of currently knowledge. Int. J. Poult. Sci. 6:694–704.

Cho, I. and Blaser, M. J. 2012. The human microbiome: At the interface of health and disease. Nat. Rev. Genet. 13:260–270.

Cho, I., Yamanishi, S., Cox, L., Methé, B.A., Zavadil, J., Kelvin, L., Gao, Z., Mahana, D., Raju K., Teitle, I., Li, H., Alekseyenko, A.V., and Blaser, M.J. 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 488 (7413):621-626.

Chung, H., Pamp, S.J., Hill, J.A., Surana, N.K., Sanna, M., Troy, E.B., Reading, N.C., Villablanca, E.J., Wang, S., Mora, J.R., Umesaki, Y., Mathis, D., Benoist, C., David, A. and Kasper, D.L.2012. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 149:1578–1593.

Colditz, I. G. 2002. Effects of the immune system on metabolism: Implications for production and disease resistance in livestock. Livest. Prod. Sci. 75:257–268.

Colson, V., Orgeur, P., Foury, A. and Mormède, P. 2006. Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. Appl. Anim. Behav. Sci. 98:70–88.

Corrier, D.E., Hinton, A.A., and Hargis, A.B.B. 2016. Effect of Used Litter from Floor Pens of Adult Broilers on Salmonella Colonization of Broiler Chicks. Avian Diseases. 36:897–902.

Costello, E. K., Stagaman, K., Dethlefsen L., Bohannan, B.J.M. and Relman D. 2012. The Application of Ecological Theory Toward and Understanding of the Human Microbiome. Science. 336:1255–1262.

Crisol-Martínez, E., Stanley, D., Geier, M.S, Hughes, R.J. and R. J. Moore. 2017. Understanding the mechanisms of zinc bacitracin and avilamycin on animal production: linking gut microbiota and growth performance in chickens. Appl. Microbiol. Biotechnol. 101:4547–4559.

Cui, Y., Wang, S. Liu, R. Sun, Y. Zhou, and Li, Y. 2017. Age-related variations in intestinal microflora of free-range and caged hens. Front. Microbiol. 8:1–10.

Cox, L.M., Yamanishi, S., Sohn, J., Alekseyenko, A.V., Leung, J.M., Cho, I., Kim, S.G., Li, H., Gao, Z., Mahana, D., Zárate Rodriguez, J.G., Rogers, A.B., Robine, N., Loke, P., Blaser, M.J. 2014. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 154(4): 705-721

DeFronzo, R.A., Ferrannini, E., Groop, L., Henry, R.R., Herman, W.H., Holst, J.J, Hu, F.B., Kahn, C.R., Raz I., Shulman, G.I., Simonson, D.C., Testa, M.A and, Weiss R. 2015. Type 2 diabetes. Nat. Rev. 15019: 1-22.

De Passillè, A., Pelletier, G., Ménard, J., and Morisset, J. 1989. Relationships of weight gain and behavior to digestive organ weight and enzyme activities in piglets. J. Anim. Sci., 67:2921-2929.

Donaldson, E.E., Stanley, D., Hughes, R.J. and Moore, R.J. 2017. The time-course of broiler intestinal microbiota development after administration of cecal contents to incubating eggs. PeerJ. 5:e3587.

D'Eath, R.B.D., and Turner, S.P., 2009. The Welfare of Pigs. Available from: http://link.springer.com/10.1007/978-1-4020-8909-1

Duar, R.M., Burkey, T.E., Fernando, S.C., Wenzel, C.Q., Tasseva, G., Lin, X.B., Blom, J., Peterson, D.A., Walter, J., Szymanski, C.M. and Frese, S.A. 2017. Experimental evaluation of host adaptation of Lactobacillus reuteri to different vertebrate species. Appl. Env. Microb. 83:e0013-17

Fagundes, C.T., Amaral, F.A., Vieira, A.T., Soares, A.C., Pinho, V., Nicoli J.R., Vieira, L.Q., Teixeira, M.M. and Souza, D.G. 2012. Transient TLR Activation Restores Inflammatory Response and Ability To Control Pulmonary Bacterial Infection in Germfree Mice. J. Immunol. 188:1411–1420.

Ferrario, C., Alessandri, G., Mancabelli, L., Gering, E., Mangifesta, M., Milani, C., Lugli, G. A., Viappiani, A., Duranti, S., Turroni, F., Ossiprandi, M. C., Hiyashi, R., Mackie, R., van Sinderen, D. and Ventura, M. 2017. Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses. Environ. Microbiol. 19:4771–4783.

Foditsch, C., Van Vleck Pereira, R., Ganda, E.K., Gomez, M.S., Marques, E.C., Santin T. and Bicalho, R.C. 2015. Oral administration of Faecalibacterium prausnitzii decreased the incidence of severe diarrhea and related mortality rate and increased weight gain in preweaned dairy heifers. PLoS One. 10:1–17.

Forder, R.E.A., Howarth, G.S., Tivey, D.R. and Hughes, R.J. 2007. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. Poult. Sci. 86:2396–2403.

Foster, K.R., Schluter, J., Coyte, K.Z., and Rakoff-Nahoum, S. 2017. The evolution of the host microbiome as an ecosystem on a leash. Nature. 548:43–51.

Frese, S.A, Hutkins, R.W. and Walter, J. 2012. Comparison of the Colonization Ability of Autochthonous and Allochthonous Strains of Lactobacilli in the Human Gastrointestinal Tract. Adv. Microbiol. 02:399–409.

Fujimura, K.E., Sitarik, A.R., Havstad, S., Panzer, A.R., Boushey, H.A., Lukacs, N.W., Wegienka, G.R., Woodcroft, K.J., Zoratti, E.M., Ownby, D.R., Levin, A.M., Johnson, C.C. and

Lynch, S.V. 2015. Development of childhood atopy is associated with early-life gut microbial enterotypes. Am J Respir Crit Care Med. 191:2186.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., Takahashi, M., Fukuda, N.N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, J.M, Topping, D.L., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K., Hase, K. and Ohno H. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 504:446–450.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., and Hinton, J.C.2006.Butyrate specifically down-regulates Salmonella Pathogenicity Island 1 Gene Expression. App. Environ. Microbiol. 72:946–949.

Gao K., Pi Y., Peng Y., Mu C.L. Zhu W.Y. 2018. Time-course responses of ileal and fecal microbiota and metabolite profiles to antibiotics in cannulated pigs. Appl. Microbiol. and Biotech. 102:1189-1199

Gilbert, M., Xiao, X., and Robinson, T. P. 2018. Intensifying poultry production systems and the emergence of avian influenza in China: a “One Health/Ecohealth” epitome. Arch. Pub. Health. 75:48.

Gomes, A.M.P. and F.X.Malcata. 1999. Bifidobacterium spp. and Lactobacillus acidophilus: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci. Technol. 10:139–157.

Gresse, R., Chaucheyras-Durand, F., Fleury, M.A., Van de Wiele, T., Forano, E. and S. Blanquet-Diot, 2017. Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol. 25: 851-873.

Guillemet R., Hamard, A., Quesnel, H., Père, M.C., Etienne, M., Dourmad, J.Y. and Meunier-Salaün M.C. 2007. Dietary fibre for gestating sows: effects on parturition progress, behaviour, litter and sow performance. Animal. 1(6):872-80

Harmsen, H.J.M.,Wildeboer–Veloo, A.C.M., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G.; Welling, G.W. 2000. Analysis of Intestinal Flora Development in Breast-Fed and Formula-Fed Infants by Using Molecular Identification and Detection Methods. J. Ped. Gastroen. Nutr. 30:61-67

Holman, D.B., Brunelle, B.W., Trachsel, J. and Allen H.K. 2017. Meta-analysis To Define a Core Microbiota in the Swine Gut. mSystems. 2:e00004-17.

Hooper, L. V. 2009. Do symbiotic bacteria subvert host immunity? Nat. Rev. Microbiol. 7:367–374.

Hou, C., Liu, H., Zhang, J., Zhang, S., Yang, F., Zeng, X., Thacke, P.A., Zhang, G.and S. Qiao. 2015. Intestinal microbiota succession and immunomodulatory consequences after introduction of Lactobacillus reuteri I5007 in neonatal piglets. PLoS One. 10:1–17.

Hrncir, T., Stepankova, R., Kozakova, H., Hudcovic, T. and Tlaskalova-Hogenova, H. 2008. Gut microbiota and lipopolysaccharide content of the diet influence development of regulatory T cells: Studies in germ-free mice. BMC Immunol. 9:1–11.

Hviid, A., Svanström, H. and Frisch, M. 2011. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 60:49–54.

Ilina, L.A., Yildirim, E.A., Nikonov, I.N., Filippova, V.A., Laptev, G.Y., Novikova, N.I., Grozina, A.A., Lenkova, T.N., Manukyan, V.A., Egorov, I.A. and Fisinin, V. I. 2016. Metagenomic bacterial community profiles of chicken embryo gastrointestinal tract by using T-RFLP analysis. Dokl. Biochem. Biophys. 466:47–51.

Ipharraguerre, I., Pastor, J., Gavalda-Navarro, A., Villarroya, F., and Mereu, A. 2018. Anitmicrobial promotion of pig tissue is associated with tissue-specific remodeling bile acids signature and signalling. Sci. Rep. 8(13671):1-13.

Jacobsen, C.N., Nielsen, V.R., Hayford, A.E., Møller, P.L. Michaelsen, A., Pærregaard, B. Sandström, M. Tvede and Jakobsen M. 1999. Screening of Probiotic Activities of Forty-Seven Strains of Lactobacillus spp. by In Vitro Techniques and Evaluation of the Colonization Ability of Five Selected Strains in Humans. App. Environ. Microbiol. 65: 4949-4956.

Jackson, K.M. and Nazar, A.M. 2006. Breastfeeding , the Immune Response , and Long-term Health. J. Am. Osteopath. Assoc. 106:203–207.

Janzcyk, P., Pieper, R., Souffrant, W.B., Bimczok, D., Rothkotter, H.J. and Smidt, H. 2007. Parenteral long-acting amoxicillin reduces intestinal bacterial community diversity in piglets even 5 weeks after the administration. ISME Journal 1: 180-183.

Jensen, P. and Recén, B. 1989. When to wean — Observations from free-ranging domestic pigs. App. Anim. Behav. Sci. 23: 49-60.

Jiménez, E., Fernández, L., Marín, M.L., Martín, R., Odriozola, J.M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., and Rodríguez, J.M. 2005. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr. Microbiol. 51:270–274.

Johnson, A.K., Mcglone, J.J., and Morrow-Tesch, J. L. 2001. Behavior and performance of lactating sows and piglets reared indoors or outdoors. J. Anim. Sci. 79:2571–2579.

Joyce, S.A. 2016. Bile acid modification at the host interface:potential for nutraceutical and pharmaceutical interventions in host health. Annu. Rev. Food Science. 7:313-333.

Ju, T., Shoblak, Y., Gao, Y., Yang, K., Fouhse, J., So, Y. and Willing, B. 2017. The initial gut microbial composition is a key factor driving host responses to antibiotic treatment. Appl. Env. Microb. 83:e01107-17.

Ju, T., Kong, J.Y., Stothard, P., Willing, B.P. 2019. Defining the role of Parasutterella, a previously uncharacterized member of the core gut microbiota. ISME Journal. (in press)

Kers, J.G., Velkers, F.C., Fischer, E.A., Hermes, G.D.A, Stegeman, J.A. and Smidt, H. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. Front. Microbiol. 9:1–14.

Kogut, M. H., and Arsenault, R. J. 2017. Immunometabolic phenotype alterations associated with the induction of disease tolerance and persistent asymptomatic infection of Salmonella in the chicken intestine. Front. Immunol. 8:1–7.

Kohl, K.D., Skopec, M.M. and Dearing, M.D. 2014. Captivity results in disparate loss of gut microbial diversity in closely related hosts. Conserv. Physiol. 2:1–11.

Konstantinov, S. R., Awati, A. A., Williams, B. A., Miller, B. G., Jones, P., Stokes, C. R., Akkermans, A. D. L., Smidt, H. and Vos, W.M. 2006. Post-natal development of the porcine microbiota composition and activities. Environ. Microbiol. 8: 1191-1199.

Korver, D. R. 2006. Overview of the Immune Dynamics of the Digestive System. J. App. Poultry Res. 15: 123-135.

Kraehenbuhl, J.P., Pringault, E., and Neutra, M.R. 2010. Review article: Intestinal epithelia and barrier functions. Aliment. Pharmacol. Ther. 11:3–9

Lee, I.K., Gu, M.J., Ko, K.H., Bae, S., Kim, G., Jin, G.D, Kim, E.B, Kong, Y.Y., Park, T.S., Park, B.C., Jung, H.J., Han, S.H., and Yun, C.H. 2018. Regulation of CD4+CD8-CD25+ and CD4+CD8+CD25+ T cells by gut microbiota in chicken. Sci. Rep. 8:1–11.

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., and Gordon, J.I. 2008. Evolution of mammals and Their Gut Microbes. Science. 320:1647–1652.

Li, G., Yao, W. and Jiang H. 2014. Short-chain fatty acids enhance adipocytes differentiation in the stromal of vascular fraction of porcine tissue. J. Nut. 144: 1887-95

Li, J., Yang, K., Ju, T., Ho, T., McKay, C.A., Gao, Y., Forget, S.K., Gartner, S.R., Field C.J., Chan, C.B. and Willing, B.P., 2017. Early life antibiotic exposure affects pancreatic islet development and metabolic regulation. Sci. Rep. 7 (41778):1-12.

Lopez-Jaramillo P., Arbeláez D.G., López J.L., López, C., Martínez-Ortega, J. Rodríguez, A. G., Cubillos, S. T. 2014. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Horm Mol Biol Clin Investig. 18(1):37-45

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. and Knight, R. 2012. Diversity, stability, and resilience of the human gut microbiota. Nature. 489(7415):220-230.

Lupp, C., Robertson, M.L., Wickham, M.E., Sekirov, I., Champion, O.L., Gaynor, E.C. and Finlay, B.B. 2007. Host-Mediated Inflammation Disrupts the Intestinal Microbiota and Promotes the Overgrowth of Enterobacteriaceae. Cell Host Microbe. 2:119–129.

Maldonado-Gómez, M.X., Martínez, I., Bottacini, F., O'Callaghan, A., Ventura, M., van Sinderen, D., Hillmann, B., Vangay, P., Knights, D., Hutkins, R. W. and Walter, J. 2016. Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. Cell Host Microbe. 20:515–526.

Martin, C., Ling, P. and Blackburn, G. 2016. Review of infant feeding: Key features of breast milk and infant formula. Nutrients 8: 279.

Matamoros, S., Gras-Leguen, C., Le Vacon, F., Potel, G., and De La Cochetiere, M.F. 2013. Development of intestinal microbiota in infants and its impact on health. Trends Microbiol. 21:167–173.

Mazmanian, S. K., Liu, C.H., Tzianabos, A.O. and Kasper, D.L. 2005. An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System. Cell 122: 107-118.

Menendez, A., Willing, B.P., Montero, M., Wlodarska, M., x So, M., Bhinder, G., Vallance, B. A. and Finlay, B.B. 2013. Bacterial stimulation of the TLR-MyD88 pathway modulates the homeostatic expression of ileal paneth cell α-defensins. J. Innate Immun. 5:39–49.

Miquel, S., Martin, R., Bridonneau, C., Robert, V., Sokol, H., Bermúdez-Humarán, L. G., Thomas, M. and Langella, P. 2014. Ecology and metabolism of the beneficial intestinal commensal bacterium Faecalibacterium prausnitzii. Gut Microbes. 5:146–151.

Miller, B., Newby, T.J., Stokes, C.R., Hampson, D. and Bourne, F.J. 1983Ann Rech Vet, 14:487-492.

Mormède, P. and Hay, M. Behavioural changes and adaptation associated with weaning. In: Pluske, J.R., Le Dividich, J., Verstegen, M.W.A. (ed.). Weaning the pig: concepts and consequences. Netherlands:Wagening Academic Publishers, 2003. chap.4. p. 51-59.

Mulder, I.E., Schmidt, B., Stokes, C.R., Lewis, M., Bailey, M., Aminov, R.I., Prosser, J.I., Gill, B.P., Pluske, J.R., Mayer, C.D., Musk C.C, and Kelly, D. 2009. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biology 7: 79.

Newburg, D.S. 2000.Oligosaccharides in human milk and bacterial colonization. J Pediatr Gastroenterol Nutr, 30:S8-S17.

Notterpek, L., Roux, K.J., Amici, S.A., Yazdanpour, A., Rahner, C. and Fletcher. B.S., 2001. Peripheral myelin protein 22 is a constituent of intercellular junctions in epithelia. Proc. Natl. Acad. Sci. 98:14404–14409.

Nurmi, E. and Rantala, M., 1973. New aspects of Salmonella infection in broiler production. Nature. 241:210-211.

Odle, J., Zijlstra, R.T. and Donovan, S.M. 1996. Intestinal Effects of Milkborne Growth Factors in Neonates of Agricultural Importance. J. Anim. Sci. 74:2509–2522.

Ott, S.J., Musfeldt, M., Wenderoth, D.F., Hampe, J., Brant, O., Fölsch, U.R., Timmis, K.N. and Schreiber, S. 2004. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut. 53:685–693.

Parry, S. H., W. D. Allen, and P. Porter. 1977. Intestinal immune response to E. coli antigens in the germ-free chicken. Immunology. 32:731–41.

Pedroso, A.A. 2009. Which came first: the egg or its microbiota? Poult. Inf. Prof. 1–5.

Perez-Muñoz, M.E., Arrieta, M.C., Ramer-Tait, A.E. and Walter, J. 2017. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: Implications for research on the pioneer infant microbiome. Microbiome. 5:1–19.

Pi, Y., Gao, K., Peng, Y., Mu, C.L., Zhu, W.Y. 2019. Antibiotic-induced alterations of the gut microbiota and microbial fermentation in protein parallel the changes in host nitrogen metabolism of growing pigs. Animal. 13(2):262-272

Pluske, J.R., Hampson, D.J., and Willians, I.H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci., 51:215-236.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. and Medzhitov, R. 2004. Recognition of Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis. Cell 118: 229-241.

Rosshart, S.P., Vassallo, B.G., Angeletti, D., Hutchinson, D.S., Morgan, A.P., Takeda, K., Hickman, H.D., McCulloch, J.A., Badger, J.H., Ajami, N. J., Trinchieri, G., Pardo-Manuel de Villena, F., Yewdell, J. W., and Rehermann, B. 2017. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell. 171:1015–1028.e13.

Round, J. L., and Mazmanian, S.K. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9:313–323.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., Tuohy, K., 2018. Gut microbiota functions: metabolism and other food components. Eur J Nutr. 57:1-24.

Sansonetti, P. J. 2004. War and peace at mucosal surfaces. Nat. Rev. Imm. 4: 953-964.

Savage, D. C. 1977. Microbial Ecology of the Gastrointestinal Tract. Annu. Rev. Microbiol. 31:107–133.

Schachtschneider, K.M., Yeoman, C.J., Isaacson, R.E., White, B.A., Schook, L.B. and Pieters, M. 2013. Modulation of Systemic Immune Responses through Commensal Gastrointestinal Microbiota. PLoS One. 8.

Schamberger, G. P., and Diez-Gonzalez. F., 2002. Selection of recently isolated colicinogenic Escherichia coli strains inhibitory to Escherichia coli O157:H7. J. Food Prot. 65:1381–1387.

Schokker, D., Jansman, A.J.M., Veninga, G., de Bruin, N., Vastenhouw, S.A., de Bree, F.M., Bossers, A., Rebel, J.M.J. and Smits. M.A. 2017. Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. BMC Genomics. 18:1–14.

Schokker, D., Zhang, J., Zhang, L.L., Vastenhouw, S.A., Heilig, H.G.H.J., Smidt, H., Rebel, J. M.J., and Smits M.A. 2014. Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One. 9. doi:10.1371/journal.pone.0100040.

Seed, P.C. 2015. The human mycobiome. Cold Spring Harb. Perspect. in Med. 5: a019810.

Scupham, A. J., Patton, T. G., Bent, E. and Bayles, D. O. 2008. Comparison of the cecal microbiota of domestic and wild turkeys. Microb. Ecol. 56:322–331.

Shin, R., Suzuki, M., and Morishita, Y. 2002. Influence of intestinal anaerobes and organic acids on the growth of enterohaemorrhagic Escherichia coli O157:H7. J. Med. Microbiol. 51:201-206.

Smith, K., McCoy, K.D. and Macpherson, A.J. 2007. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin. Immunol. 19:59–69.

Souza, D.G., Vieira, A.T., Soares, A.C., Pinho, V., Nicoli, J.R., Vieira, L.Q., and Teixeira, M.M. 2004. The Essential Role of the Intestinal Microbiota in Facilitating Acute Inflammatory Responses. J. Immunol. 173:4137–4146.

Souza, M.R., Moreira, J.L., Barbosa, F.H.F., Cerqueira, M.M.O.P., Nunes, Á.C. and Nicoli, J.R. 2007. Influence of intensive and extensive breeding on lactic acid bacteria isolated from Gallus gallus domesticus ceca. Vet. Microbiol. 120:142–150.

Stanley, D., Geier, M.S., Hughes, R.J., Denman, S.E. and Moore, R.J. 2013. Highly variable microbiota development in the chicken gastrointestinal tract. PLoS One. 8:6–12.

Stanley, D, Hughes, R.J., Geier, M.S. and Moore R.J. 2016. Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: Challenges presented for the identification of performance enhancing probiotic bacteria. Front. Microbiol. 7:1–13.

Sun, J., Wang, Y., Li, N., Zhong, H., Xu, H., Zhu, Q., and Liu Y. 2018. Comparative Analysis of the Gut Microbial Composition and Meat Flavor of Two Chicken Breeds in Different Rearing Patterns. Biomed Res. Int. 2018:4343196. doi:10.1155/2018/4343196.

Tamburini, S., Shen, N., Wu, H.C. and Clemente, J.C. 2016 The microbiome in early life: implications for health outcomes. Nat. Med.. 22(7): 713-722.

Tanaka, M. and Nakayama, J. 2017. Development of the gut microbiota in infancy and its impact on health in later life. Aller. Inter. 55:515-522.

Ulrich-Lynge, S.L., Dalgaard, T.S., Norup, L.R., Kjærup, R.M., Olsen, J.E., Sørensen, P. and Juul-Madsen, H. R. 2015. The consequence of low mannose-binding lectin plasma concentration in relation to susceptibility to Salmonella Infantis in chickens. Vet. Immunol. Immunopathol. 163:23–32.

Ushida, K., Tsuchida, S., Ogura, Y., Toyoda, A. and Maruyama, F. 2016. Domestication and cereal feeding developed domestic pig-type intestinal microbiota in animals of suidae. Anim. Sci. J. 87:835–841.

Val-Laillet, D., Besson, M., Guérin, S., Coquery, N., Randuineau, G., Kanzari, A., Quesnel, H., Bonhomme, N., Bolhuis, J.E., Kemp, B., Blat, S., Le Huërou-Luron I. and Clouard, C. 2017. A maternal Western diet during gestation and lactation modifies offspring's microbiota activity, blood lipid levels, cognitive responses, and hippocampal neurogenesis in Yucatan pigs. FASEB J. 31(5):2037-2049

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T. P., Teillant, A., and Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. 112:5649–5654.

Vineetha, P.G., Tomar, S., Saxena, V.K., Kapgate, M., Suvarna, A.and Adil.,K. 2017. Effect of laboratory-isolated Lactobacillus plantarum LGFCP4 from gastrointestinal tract of guinea fowl on growth performance, carcass traits, intestinal histomorphometry and gastrointestinal microflora population in broiler chicken. J. Anim. Physiol. Anim. Nutr. (Berl). 101:e362–e370.

Wang, P., Geng, H., Bu, H.F., Liu F., Wang, X., Kurowski, J., Akhtar, S., Li, Y.Q., and Tan X.D. 2015. Down-Regulation of Peripheral Myelin Protein 22 (PMP22) Contributes to Impairment of Intestinal Barrier Integrity in Sepsis: Role of TNF-α and IFN-γ. Gastroenterology. 148:S-85.

Wang B., Yao M., Lv L., Ling Z.,Li L. 2017. The human microbiota in health and disease. Engineering 3:71-82.

Wei, H.K., Xue, H.X., Zhou, Z.X., and Peng, J. 2017. A carvacrol-thymol blend decreased intestinal oxidative stress and influenced selected microbes without changing the messenger RNA levels of tight junction proteins in jejunal mucosa of weaning piglets. Animal. 11:193–201.

Wei, S., Gutek, A., Lilburn, M., and Yu, Z. 2013. Abundance of pathogens in the gut and litter of broiler chickens as affected by bacitracin and litter management. Vet. Microbiol. 166:595–601.

Willing, B. P., and Van Kessel, A.G. 2007. Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. J. Anim. Sci. 85:3256–3266. doi:10.2527/jas.2007-0320.

Willing, B.P., Vacharaksa, A., Croxen, M., Thanachayanont, T., and Finlay, B.B.. 2011b. Altering host resistance to infections through microbial transplantation. PLoS One. 6:2–10.

Willing, B.P., Russell, S.L., and Finlay, B.B. 2011b. Shifting the balance: Antibiotic effects on host-microbiota mutualism. Nat. Rev. Microbiol. 9:233–243.

Winter, S.E., Winter, M.G., Xavier, M.N., Thiennimitr, P., Poon, V., Keestra, A.M., Laughlin, R.C, Gomez, G., Wu, J., Lawhon, S.D, Popova, I.E., Parikh, S.J., Adams, L.G., Tsolis, R.M. Stewart, V.J. and A.J. Bäumler. 2013. Host-derived nitrate boosts growth. Science. 339:708–711.

Wlodarska, M., Willing, B., Keeney, K.M., Menendez, A., Bergstrom, K.S., Gill, N., Russell, S. L., Vallance, B.A., and Finlay B.B. 2011. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated Citrobacter rodentium-induced colitis. Infect. Immun. 79:1536–1545.

Yan H., Lu H., Almeida, H.H., Ward, M.G., Adeola, O., Nakatsu, C.H., and Ajuwon K.M. 2015. Effects of dietary resistant starch content on metabolic status, milk composition, and microbial profiling in lactating sows and on offspring performance. J. An. Phys. Nut. 1-11.

Yang, L., Liu, S., Ding, J., Dai, R., He, C., Xu, K., Honaker C.F., Zhang, Y., Siegel, P. and Meng H. 2017. Gut microbiota co-microevolution with selection for host humoral immunity. Front. Microbiol. 8:1–11.

Youssef, N.H., Couger, M.B., McCully, A.L., Criado, A.E.G., and Elshahed, M.S. 2015. Assessing the global phylum level diversity within the bacterial domain: A review. J. Adv. Res. 6:269–282.

Zheng, L., Kelly, C.J., Battista, K.D., Schaefer, R., Lanis, J.M., Alexeev, E.E., Wang, R.X., Onyiah, J.C., Kominsky, D.J. and Colgan, S.P. 2017. Microbial-Derived Butyrate Promotes Epithelial Barrier Function through IL-10 Receptor–Dependent Repression of Claudin-2. J. Immunol. ji1700105. doi:10.4049/jimmunol.1700105.

Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., De Sauvage, F.J. and Ouyang, W. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14:282–289.

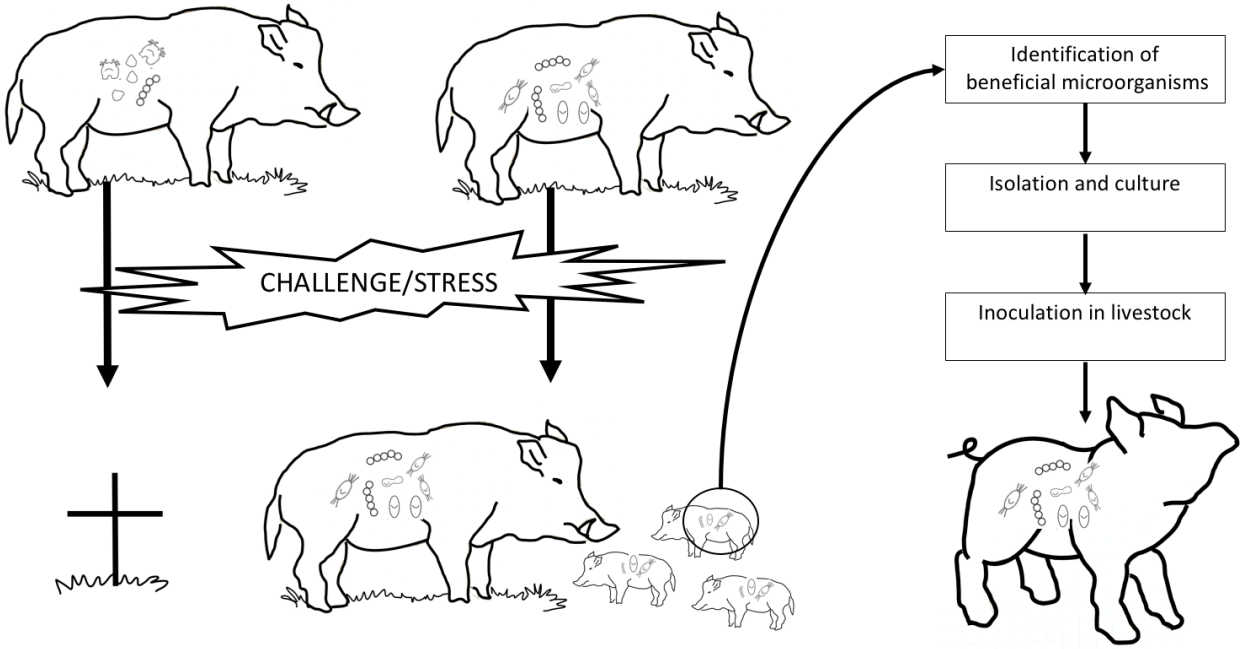


Figure 2.1. The microbiota evolves to persist within the host. It is expected that animals that harvest a beneficial microbiota will be more apt to survive in challenging situations, such as infectious diseases and food scarcity. Identification, isolation and inoculation of specific, host-adapted, beneficial organisms may be a strategy to promote disease-resistance and performance in livestock species.

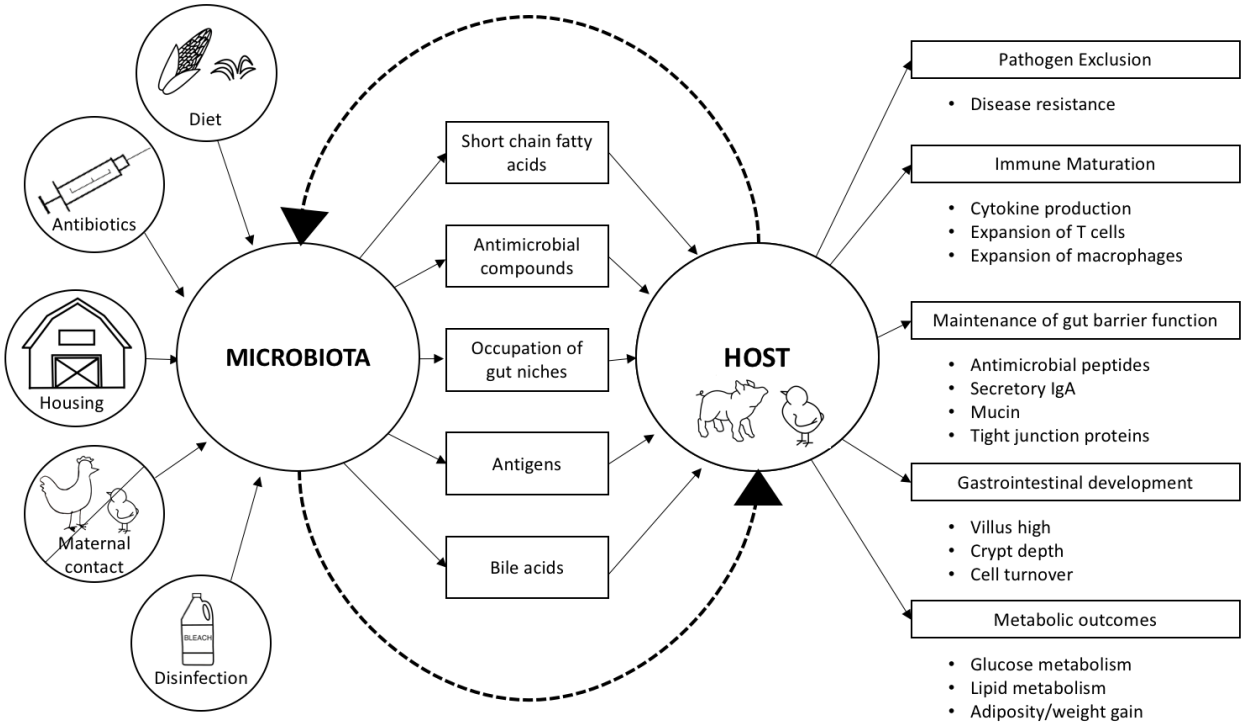


Figure 2.2. Intensive livestock production practices affect microbial community composition and function, thus impacting host physiology, metabolism, and immune responses. Microbial perturbations in early life may have life-long consequences in microbiota dynamics and host health and disease-resistance.

3. CHAPTER 3: INVESTIGATING THE CECAL MICROBIOTA OF BROILERS RAISED IN EXTENSIVE AND INTENSIVE PRODUCTION SYSTEMS

3.1. INTRODUCTION

The community of bacteria living in the gastrointestinal tract (GIT) of poultry has been shown to influence many aspects of host physiology, including nutrient digestion [1–3], immune system maturation and tunning [4–6], disease resistance and tolerance [7–9], and intestinal development [10]. Throughout evolution, this association resulted in bacteria that are highly adapted to the GIT of specific host species [11], that are likely to colonize the GIT after a single exposure [12], and that can significantly affect host metabolism [13]. Moreover, the microbiota can respond quickly to environmental conditions and promote changes in host phenotype that aid acclimation and adaptation to new situations [14, 15]. Therefore, it is expected that individuals hosting beneficial bacteria are more prone to survive, reproduce and transmit their commensal bacteria to the next generation, favoring the persistence of both the host species and beneficial commensal organisms [15, 16].

Despite growing evidence indicating the importance of proper microbial colonization [17], intensive farming practices are structured in a way that reduces opportunities for the selection and transmission of beneficial commensal bacteria across generations. In intensive production systems (IPS), broilers are hatched in artificial hatcheries and moved to enclosed barns, which limits the contact with the microbiota of mature birds. In addition, broilers in IPS are fed standardized diets with a limited number of ingredients, which may also contain antibiotics. In contrast, broilers reared in extensive production systems (EPS) might have access to outdoor environments and be fed antibiotic-free diets, which offers more opportunity to exposure and colonization by a wide

variety of microorganisms. In face of the restrictions on the use of antibiotics due to concerns about the development of antimicrobial resistance, there is a need to find alternative strategies to promote animal growth and control disease occurrence in IPS. The restoration of the chicken native microbiome using next-generation probiotics, which include bacterial strains that reside in the intestinal ecosystem, could be a potential alternative [18]. This contrasts with "traditional" probiotic products that usually contain bacterial strains harvested from soil and fermented food, which may fail to effectively colonize and persist in the chicken GIT unless being constantly provided [12, 19]. The lack of host-adaptation traits coupled with individual variabilities in broiler microbiota communities could be the reason why probiotics that aimed to improve performance and disease resistance have inconsistent results [20, 21].

Domesticated animals colonized with microbiota from wild counterparts have shown reduced inflammatory responses and increased survival following infection challenges [22]; and their microbiota was shown to be more resilient to disturbances caused by factors such as dietary and environmental changes, as well as antibiotic use [23]. Bacteria from chickens from EPS were shown to have higher antagonist activity against pathogens and less resistance to antimicrobials compared to bacteria from chickens from IPS [24, 25]. In addition, studies have shown that poultry raised in IPS may lack core bacteria that are found in free range and feral birds [26–28].

Previous studies comparing the microbiota of poultry raised in IPS and EPS presented some limitations, such as small sample size and unavailability of samples from similar aged birds from each system [25, 28]. In the present study, we characterized the cecal microbiota of age-matched broilers from 22 independent commercial farms under IPS (n = 12 farms) or EPS (n = 10 farms), aiming to determine which bacteria are normal inhabitants of the chicken GIT and which bacteria might be missing from broilers in IPS. Further genomic characterization of selected commensal

bacterial isolates has also been performed to guide the development of next-generation probiotics and guide further studies aiming at understanding the role of individual bacteria within the gut microbiota.

3.2. RESULTS

The amplicon sequencing of 105 cecal samples generated an average of 52,457 ± 36,444 (mean ± standard deviation (SD)) reads that were assigned to 12,331 ASVs and 792 taxa. Samples were rarefied at 11,360 reads, resulting in removal of 4,172 ASVs and 100 taxa that were not present in any sample after random subsampling. Two samples that presented less than 11,360 reads were excluded from downstream analysis. Analyses of 103 rarefied samples (n = 45 from EPS and n = 58 from IPS) indicated a presence of 8,159 ASVs and 692 taxa.

3.2.1. Production systems significantly affect cecal microbiota composition

The cecal microbiota of broilers raised in EPS had higher phylogenetic diversity than that of birds in IPS ($p < 0.001$) (Figure 1A); but no significant differences were observed in alpha-diversity indices including Chao1 (Figure 1B), Shannon, and Simpson (Figure S1) ($p = 0.06$, $p = 0.93$ and $p = 0.35$, respectively). PERMANOVA analysis indicated that the microbiota composition was significantly different between systems ($p = 0.001$, $R^2 = 0.10$) (Figure 1C). Hierarchical clustering analysis showed a clear separation of samples according to rearing system, except for a single sample obtained from a broiler from IPS that was clustered with samples obtained from broilers from EPS (Figure 2A). In addition, samples of birds from the same farm clustered tightly, except for 3 samples from 3 different farms that were separated from the

remaining samples obtained from the same farm (Figure S2). Bacteroidetes dominated the cecal microbial community in EPS birds (55.2% ± 8.9 (mean relative abundance ± SD)), whereas Firmicutes dominated the cecal microbial community in IPS broilers (61.7% ± 14.4) (Figure 2B). Six phyla were exclusively detected in broilers from EPS including Deferribacteres (0.8 % ± 1.1, $p < 0.001$), Elusimicrobia (0.9 % ± 3.4, $p < 0.001$), Fusobacteria (0.1 % ± 1.3, $p < 0.001$), Patescibacteria (0.3% ± 0.8, $p < 0.001$), Spirochaetes (2.7 % ± 4.1, $p < 0.001$), and Synergistetes (0.4 % ± 0.5, $p < 0.001$). Besides the six unique phyla, the cecal microbiota of EPS broilers presented higher relative abundance of Actinobacteria (1.2% ± 0.9 *vs*. 0.7% ± 1.1, $p < 0.001$), Bacteroidetes (55.2 % ± 8.9 *vs.* 27.9% ± 11.9, $p < 0.001$), Lentisphaera (0.5% ± 0.5 *vs.* 0.01% ± 0.04, $p < 0.001$), Proteobacteria (4.7% ± 3.0 *vs*. 0.3% ± 1.0), $p = 0.002$) and Verrucomicrobia (0.7% ± 1.3 *vs*. 0.3 ± 1.0, $p < 0.001$) compared with that in IPS broilers. On the other hand, IPS broilers had enriched Firmicutes (61.7% ± 14.4 *vs.* 28.7% ± 7.5, $p < 0.001$) and Tenericutes (1.0% ± 1.1 *vs.* 0.4% ± 0.6, $p = 0.003$). The most abundant phyla within the microbiota of broilers in both systems were Firmicutes, Bacteroidetes and Proteobacteria (Figure S3).

3.2.2. EPS cecal microbiotas harbor unique ASVs

Among the total number of ASVs detected, 52.5% were found to be unique to birds raised in EPS, 33.4% were unique to birds raised in IPS, and 14.3% were shared between both systems. An average of 67.8% ± 14.4 (mean relative abundance ± SD) of the cecal microbial community in EPS broilers was composed of ASVs that were unique to this system, whereas the ASVs unique to the microbiota of IPS broilers composed only 14.5% ± 6.7 of the cecal microbial community in IPS broilers (Figure S4A). We further looked at phyla assignment for shared and unique ASVs and found that Bacteroidetes was the most abundant phyla detected within the unique microbial

members of extensively and intensively raised broilers composing 71.2% ± 10.9 of the unique community of EPS and 46.6% ± 22.9 of the unique community in IPS (Figure S4B). Most of the ASVs shared by both systems were Firmicutes, with relative abundance of 73.7% ± 17.4 in EPS and 72.5% ± 15.0 in IPS (Figure S4C).

Differential abundance analyses at the ASV level were performed after aggregating all ASVs not seen in more than 20 birds into a single feature named "Rare". This reduced the number of ASVs from 8,153 to 452 ASVs that represented an average relative abundance of 72.6% ± 9.5 (mean ± SD) of the microbial community in IPS birds and 35.5% ± 9.5 of the community in EPS. Within these 452 ASVs, 158 and 33 ASVs were enriched in broilers from IPS and EPS, respectively. ASVs that were aggregated as "Rare" were enriched and composed most of the microbial community (64.5% ± 9.5) in EPS birds. The microbiota of IPS birds was mostly composed of ASVs that were not differentially abundant between the systems (39.8% ± 12.3). It was also noteworthy that ASVs that were enriched in IPS birds were consistently found in EPS birds with an average relative abundance reaching 16.5% ± 9.0 in the latter; whereas the ASVs enriched in EPS birds rarely occurred in IPS broilers, which had an average relative abundance of only 0.6% ± 0.8 in the latter (Figure 3, Table S1). Without performing aggregation of rare ASVs, differential abundance analyses resulted in 249 ASVs enriched in EPS broilers and 130 ASVs enriched in IPS broilers.

3.2.3. The family Enterobacteriaceae is enriched in IPS microbiotas

Differential abundance analyses at the family level were performed after combining families not detected in more than 20 birds into a single category, called "Rare", which reduced

the number of bacterial families from 97 to 53. Analyses of these 53 families indicated that five bacterial families were unique to the microbiota of EPS broilers, namely *Deferribacteraceae* (0.8% ± 1.0, $p < 0.001$), *Elusimicrobiaceae* (0.9% ± 3.4, $p < 0.001$), *Spirochaetaceae* (2.7% ± 4.0, $p < 0.001$), *Synergistaceae* (0.4% ± 0.5, $p < 0.001$), and *Victivalles vadin BE97* (0.1% ± 0.2, $p < 0.001$). In addition, nine families were found to be enriched in EPS birds, whereas seven families were enriched in IPS birds including *Enterobacteriaceae* ($p < 0.001$). The average relative abundance of *Enterobacteriacea* in IPS and EPS broilers was 2.4% ± 3.9 and 0.2% ± 0.3 (Figure S5).

3.2.4. IPS cecal microbiotas are missing microbes

At the taxonomic level, 49.4% of the total number of taxa was shared between the cecal microbiota of broilers from both systems, whereas 41.5% and 9.1% of taxa were unique to the cecal microbiota of broilers raised in EPS and IPS, respectively. Analyses of features assigned to the genus and species levels indicated ten genera and six species to be missing from the microbiota of EPS broilers; whereas 55 genera and 31 species were indicated to be missing from the microbiota of IPS broilers (Table S2). All taxa found to be missing from EPS broilers presented rare occurrence in IPS broilers and the same was true for most of the taxa found to be missing from IPS birds. However, some taxa commonly found in EPS birds were completely absent from IPS birds, namely the species *Bacteroides plebeius* (2.3% ± 3.9 (mean relative abundance ± SD) , $p < 0.001$), *Bacteroides salanitronis* (0.2% ± 0.4, $p < 0.001$), as well as genera *Alloprevotella* (2.2% ± 2.9, $p < 0.001$), *Prevotellaceaee UCG-001* (1.6% ± 0.8), *Mucispirillum* (0.8% ± 1.1, $p < 0.001$), *Elusimicrobium* (0.9% ± 3.4, $p < 0.001$), and *Synergistes* (0.4% ± 0.5, $p < 0.001$).

Differential abundance analyses at the taxa level were performed after combining all taxa present in less than 20 birds as a single taxon called "Rare", which reduced the number of taxa from 692 to 241. We found 39 taxa enriched in EPS birds, and 36 taxa enriched in IPS birds. Most of the differentially abundant taxa showed an average relative abundance below 0.5% (Figure 4, Figure S6). A total of 164 taxa were assigned as core components of the broiler cecal microbiota with 90 of which were shared between birds in both systems (Figure S7). In addition, 44 taxa were assigned as core in EPS birds and 31 taxa were assigned as core in IPS birds (Figure 5). The cecal microbiota of IPS broilers was shown to be depleted of taxa that were core members of EPS birds, such as *Olsenella* (0.3% ± 0.22 *vs.* 0.01% ± 0.03 ($p = 0.001$), *Bacteroidales* (38.1% ± 3.0 *vs*. 20.7% ± 1.9, $p < 0.001$), *Bacteroides gallinaceum* (2.6% ± 4.2 *vs.* 0%, $p < 0.001$), *Bacteroides plebeius* (2.3% ± 3.9 *vs.* 0%, $p < 0.001$), *Muribaculaceae* (1.3% ± 1.9 *vs.* 0%, $p < 0.001$), *Parabacteroides* (0.9% ± 0.7 *vs.* 0.4% ± 0.9, $p < 0.001$), *Prevotellaceae*-UCG-001 (1.6% ± 0.8 *vs*. 0%, $p < 0.001$), *Rikenellaceae* RC9 (9.2% ± 7.6 *vs.* 0%, $p < 0.001$), *Mucispirillum* (0.8% ± 1.1 *vs.* 0%, $p < 0.001$), *Elusimicrobium* (0.9% ± 3.4 *vs.* 0%, $p < 0.001$), *Victivallaceae* (0.1% ± 0.2 *vs*. 0%, $p < 0.001$), *Desulfovibrio* (0.4% ± 0.5 *vs.* 0.0 ± 0.1%, $p < 0.001$), *Sutterella* (0.9% ± 0.8 *vs.* 0.1% ± 0.4, $p < 0.001$), *Synergistes* (0.4% ± 0.5 *vs.* 0%, $p < 0.001$) and *Puniceicoccaceae* (0.2% ± 0.2 *vs.* 0%, $p < 0.001$) The microbiota of EPS birds presented a lower frequency of Firmicutes that were core members of IPS birds; although complete depletion was not observed for any taxa in EPS birds.

3.2.5. The EPS microbiota has greater predicted functional potential

Principal component analysis of predicted Enzyme Commission genes and MetaCyc pathways indicated a clear separation between samples obtained from EPS and IPS broilers (Figure

S8). A total of 167 pathways were shown to be differentially present, from which 95 were enriched in EPS broilers, and 52 were enriched in IPS broilers. With an effect size of 0.5 as a threshold, 60 pathways were identified as biologically relevant (Figure 6). A total of 75 ASVs were estimated to contribute to the enriched pathways, and most of the contributing ASVs were assigned to the order Clostridiales (Table S3). The microbiota of EPS broilers presented 53 enriched pathways which were mainly involved in the biosynthesis of amino acids (L-arginine, L-serine, L-tyrosine), cofactors and vitamins (B6, B9, B12, K2, coenzyme A), fatty acids, and carbohydrates. In addition, the microbiota of EPS broilers showed several enriched pathways related to nutrient degradation and assimilation, including rhamnose degradation and sulfur assimilation, and pathways related to the generation of precursor metabolites such as methane and propionic acid. On the other hand, only seven pathways were shown to be enriched in the microbiota of IPS broilers. These pathways were shown to be involved in the biosynthesis of L-methionine, dTDP-N-acetylthomosamine (an important antigen in the outer membrane of E*nterobacteriaceae*), and heptose sugars (commonly found in the cell surface of many bacteria).

3.2.6. Isolation of chicken commensals and whole genome sequencing (WGS)

We collected and identified 410 isolates, which were assigned to 87 species from six phyla. Most isolates were members of Firmicutes, comprising 53 species; followed by 14 species from Bacteroidetes, 10 from Actinobacteria, 7 from Proteobacteria, 2 from Cyanobacteria and one species from Fusobacteria (Figure 7, Table S4). We selected 24 isolates from phyla Actinobacteria, Bacteroidetes, and Firmicutes for WGS analysis, from which 5 were considered as new species. The isolates considered as new species had low OrthoANI values compared to reference genome sequences of *[Collinsella] massiliensis* (75.93%), *Bacteroides gallinaceum* (74.2%), *Bacteroides*

uniformis (75.03%), *Barnesiella viscericola* (85.73%) and *[Ruminococcus] torques* (74.3%) (Table S5). The presence of genes encoding bacteriocins and identified virulence factors are indicated in Tables S6 and S7. The presence of antibiotic resistance genes is illustrated in Figure 8.

3.3. DISCUSSION

Identifying bacteria that are normal inhabitants of the cecal microbiota of broilers is an initial step to use microbial manipulation in favor of broiler production. As intensive production practices may hinder the transmission of commensal microbes across generations [29], exploring potential missing microbes in intensively raised broilers compared with broilers reared in extensive systems can guide the development of microbial strategies to manipulate broiler health and performance. In the current study, characterized the cecal microbiota of market-aged broilers raised in 22 independent commercial farms under intensive and extensive production systems. We found that the microbiota of EPS broilers was significantly different and had higher phylogenetic diversity than the microbiota of IPS broilers. Numerous bacteria were completely absent from IPS broilers compared with EPS broilers. Among these missing bacteria, some were core in broilers from EPS, including *Olsenella*, *Bacteroides gallinaceum*, *Bacteroides plebeius*, *Parabacteroides*, *Mucispirillum*, *Elusimicrobium*, *Victivallaceae*, *Sutterella*, *Desulfovibrio*, and *Synergistes*. On the other hand, a few missing bacteria, such as fusobacteria and patescibacteria, were only occasionally observed in EPS broilers and therefore considered less relevant.

At the phylum level, there was a clear difference between the systems, with the cecal microbiota of EPS broilers presenting a higher relative abundance of Bacteroidetes and

Proteobacteria, while the cecal microbiota of IPS broilers was dominated by Firmicutes. This was consistent with previous studies comparing poultry in extensive and intensive systems [25, 27, 30–33], and has also been observed in humans living in rural and urbanized societies [34, 35]; as well as in wild and captive rodents [22, 36]. The similarity amongst results is not surprising since broilers are captive animals and current production practices resemble the changes in lifestyle that human society underwent during urbanization, which includes sanitation, use of antibiotics, and reduced microbiota transmission between mothers and offspring [37].

Previous studies have indicated that spore-forming and aerotolerant bacteria, such as most Firmicutes and Proteobacteria, are likely acquired from the environment; whereas bacteria not equipped to survive in aerobic conditions, such as Bacteroidetes (non-spore forming strict anaerobes), need to be transmitted from mother to the offspring [38]. EPS broilers are more likely to present higher Bacteroidetes abundance due to their contact with a less sanitized environment and potential encounter with fecal material from mature birds; while IPS broilers are more likely to be colonized by Firmicutes present in the environment. Non-spore forming strict anaerobes present higher colonization capacity and a higher degree of host adaptation than that of spore-formers and aerotolerant bacteria [12]. In that way, the reduction of Bacteroidetes and other obligate anaerobes such as *Megamonas* and *Parasutterella* indicates that intensively raised broilers are missing host-adapted species that likely co-evolved with birds in nature.

Among enriched Bacteroidetes species in the cecal microbiota of EPS broilers, species *Alistipes*, *Barnesiella*, *and Parabacteroides* have been shown to be efficient colonizers of the broiler ceca [39, 40] and to be enriched in chicks raised in contact with an adult hen [41]. Specifically, *Alistipes*, one of the first colonizers of the chicken GIT [42], has been positively correlated with broiler body weight [43], and shown to be more abundant in the ceca of healthy

broilers compared to coccidiosis infected broilers [33] and in the ceca of free-range chickens compared to commercial broilers [28]. *Barnesiella* has been shown to be enriched in the ceca of free-range chickens [28], high performing broilers [33] and chicks colonized with cecal material [40]. *Parabacteroides* has been shown to be positively correlated with broiler body weight [44] and to be more abundant in older chickens [28, 45–47], which could be an indicator of a more mature microbiota.

Within the genus *Bacteroides*, *Bacteroides thetaiotaomicron* and *Bacteroides fragilis* were enriched in the microbiota of IPS broilers, whereas *Bacteroides salinitronis* DSM 18170, *Bacteroides plebeius*, and *Bacteroides gallinaceum* were enriched in the cecal microbiota of EPS broilers. *B. plebeius*, *B. gallinaceum* and *B. salinitronis* have been previously shown to be chicken-adapted species, whereas *B. thetaiotaomicron* has been shown to be human-adapted [48], thus indicating that IPS broilers are likely being colonized by *Bacteroides* from human origin instead of chicken commensals. The reduction of host-adapted species may affect several aspects of bird physiology [6, 9, 49]. For example, it was recently shown that week-old broilers with high *Bacteroides* abundance had increased short-chain fatty acid concentration, higher cecal claudin-1 and IL-10 expression, and lower expression of interleukin-1β compared to broilers with low *Bacteroides* abundance, suggesting that *Bacteroides* can promote polysaccharide degradation, improve intestinal barrier, and modulate immune responses towards downregulation of inflammatory pathways [50].

Within the phylum Firmicutes, *Megamonas*, a core member of the broiler cecal microbiota, was shown to be less abundant in IPS broilers. *Megamonas* has been shown to be an efficient colonizer of the broiler gut and to be enriched in broilers inoculated with adult cecal content and defined communities [6, 39, 41, 51, 52]. Interestingly, enriched *Megamonas* has also been

observed in wild and free-range birds compared to birds in captivity [26, 28, 53, 54]. In addition to being a free hydrogen utilizer and short-chain fatty acid producer, *Megamonas* has been shown to inhibit *Salmonella* growth *in vitro* [55, 56], which warrants further exploration of the function of this genus in the chicken GIT.

Within the phylum Proteobacteria, the cecal microbiota of EPS broilers showed higher relative abundance of *Sutterella*, *Parasutterella*, and *Desulfovibrio*. *Desulfovibrio* is an effective colonizer of the chicken ceca [39, 41] which can consume free hydrogen present in the gut environment [42]. *Parasutterella* has been identified as a core member of the gut microbiota of 35-day old broilers [46] and *Sutterella* has been positively associated with broiler body weight [44]. Although there has been no specific study evaluating the role of these genera in the gut microbiota of chickens, *Parasutterella* has been shown to significantly impact host physiology by modulating bile acid and cholesterol metabolism in mice [13].

In the current study, six phyla were exclusively detected in extensively raised birds. Among these six unique phyla, Fusobacteria was a minor component of the broiler microbiota, which agrees with other studies [42]. Despite its relative low abundance (0.1 % ± 1.3), some *Fusobacteria* species can degrade uric acid [1, 55], which could be of importance to broiler physiology. Spirochaetes were detected in 46% of the EPS broilers, with an average relative abundance of (2.7 % ± 4.1). Members from this phylum have been reported to be enriched in free range chickens [57] and Indian native chicken breeds [32] compared to commercial broilers. The main Spirochaetes detected in our study were *Treponema*, *Sphaerochaeta*, and *Brachyspira*, which have been considered as potential pathogens [58]. Some Treponema species can degrade cellulose and xylan [59], and the observed enrichment in EPS chickens could be resultant from an access to high fiber substrates. The phylum Elusimicrobia was present in 50% of the EPS broilers, with an

average relative abundance of (0.9 % ± 3.4). Elusimicrobium was previously shown to be missing from commercial broilers compared to indigenous breeds [32] and to be a core microbe in the cecal microbiota of 81-day-old free range chickens [28]. Interestingly, an increase in *Elusimicrobium* was observed in the microbiota of laying hens fed insect-based diet [60]. As a common member found in the GIT of insects [61], the presence of *Elusimicrobium* in EPS broilers is likely a consequence of these birds having access to insects as food sources. The phylum Synergistetes was detected in most EPS birds, with an average relative abundance of (0.4% ± 0.5). This phylum has been reported both as a minor and major component of the chicken gut microbiota [41, 62–65] Synergistetes species can degrade toxic compounds from plants [66] and were shown to be abundant in the ceca of wild capercaillie but absent from captive birds. Capercaillie relies on conifer plants as feed source during winter, and it is speculated that the low survival rates observed in captive birds re-introduced to wild environments is resultant from a lack of Synergistetes within the gut microbiota of captive birds [53]. The reduction in Synergistetes seen in captive birds coupled with the impaired host ability to detoxify toxic compounds and digest plant materials warrant further investigation of the impact of Synergistetes on the cecal microbiota of broilers.

The microbiota of IPS broilers had a reduced frequency of *Olsenella* and *Victivallis*. In contrast with our results, a previous study observed *Olsenella* to be increased in indoor hens compared to outdoor reared hens [25]. It is possible that the contradictory results are due to differences in *Olsenella* species present across studies. In our study, the only ASV assigned to the species level was *Olsenella* sp. Marseille-P3256, which has recently been reclassified as *Thermophilibacter mediterraneous* [67]. We were able to isolate *Thermophilibacter mediterraneous* and *Thermophilibacter provencensis,* which are former *Olsenella* species that could be employed in future studies to evaluate the impact of *Olsenella* on host physiology. In

addition, in agreement with our results, *Victivallis* was previously shown to be enriched in extensively raised chickens [25, 28], which could be related to an access to insects, as *Victivallis* was also shown to be enriched in chickens fed insect larvae [68].

The microbiota of IPS broilers showed an enrichment in *Blautia*, *Faecalibacterium*, and *Oscillibacter*, which were core microbes shared by birds in both systems. *Blautia* and *Oscillibacter* were previously observed as enriched in indoor- compared to outdoor-raised chickens [25]. Interestingly, chicks inoculated with a competitive exclusion product containing *Blautia* and *Oscillibacter* were shown to have lower relative abundance of *Blautia* than non-inoculated chicks; while no *Oscillibacter* was detected in either group [69]. This suggests that *Blautia* and *Oscillibacter*, although ubiquitous to the broiler cecal microbial community, can be displaced by other bacteria if other bacteria are available. *Faecalibacterium* is a major butyrate producer [70] that has been associated with improved growth performance and gut health in broilers [21, 33, 44, 71–73]; however, the characterization of the effect of *Faecalibacterium* on broiler physiology is still needed. In addition, several genera with the relative abundance lower than 0.5% were also shown to be enriched in the microbiota of IPS broilers. Amongst these enriched genera, *Bacillus* and *Butyricicoccus* have been previously mentioned as core members of the broiler microbiota [28, 31, 74]. Several *Bacillus* species have been used as probiotics and suggested to confer benefits to broilers [75], however, *Bacillus* species are usually not effective colonizers of the chicken gut and need to be constantly provided to exert effects [12, 41] *Butyricoccus* has been considered as a potential probiotic due to its production of butyrate [76] and positive association with broiler performance and disease resilience [72, 77]; however, it has been reported that pure cultures of *Butyricicoccus pullicaecorum* inoculated in day-old chicks failed to colonize the ceca [39].

A greater predictive functional potential observed in the microbiota of EPS broilers may be related to different dietary patterns between systems. Although greater functional potential could be considered beneficial, the pathways enriched in EPS microbiota may not necessarily benefit IPS broilers which are fed relatively simplified diets. Nonetheless, identifying and harvesting bacteria capable of providing functional potential could aid the use of alternative feed ingredients if coupled with supplementation of specific bacteria that can utilize these ingredients. Moreover, bacteria harvested from EPS chickens could potentially have lower incidence of antimicrobial resistance genes and higher ability to inhibit pathogen growth. A limitation of our results is that the accuracy of functional predictions based on 16S rRNA sequencing is dependent upon availabilities of reference genomes, which could potentially result in limited annotation of particular ASVs. Future studies using techniques such as metagenomic sequencing will help improve the accuracy of functional annotation of microbial communities in EPS and IPS chickens.

3.4. CONCLUSION

In the current study, we focused on bacteria from EPS chickens since these bacteria are more likely to be host-adapted and to have evolved with chickens in nature. We identified *Olsenella*, *Alistipes*, *Bacteroides*, *Barnesiella*, *Parabacteroides*, *Megamonas*, and *Parasutterella* as core microbes within the broiler cecal microbiota to be further investigated for their effects on bird physiology and potential applications as next-generation probiotics. These genera seem to be depleted in IPS broilers but are frequently found in EPS broilers and readily colonize the ceca after a single exposure. The collection of bacterial isolates generated in this study will be used as a resource to further explore how differences in microbiota composition can influence bird physiology and to elucidate the role of individual species within the microbial community.

3.5. METHODS

3.5.1. Samples

Farms that participated in this research project were recruited with assistance of poultry industry workshops, producer associations, and local veterinarians. Details about the research project were introduced to participated producers, and samples were collected with a research consent. Cecal samples from IPS broilers (n = 59) were collected from 12 independent commercial farms. The broilers were euthanized on farm using cervical dislocation and cecal contents were collected using sterile technique into an empty tube or a tube containing liquid casein yeast (LCY) media supplemented with 30% glycerol and 0.05% L-cysteine. Samples were transported on dry ice and stored at -80°C until use. Cecal samples from EPS broilers (n = 46) were collected from 10 independent commercial small-scale farms that supply poultry products to local farmers' markets. Among these 10 farms, one farm that raised free-range broilers in an organic system was visited and samples were collected as described for IPS broilers. Samples from the remaining nine EPS farms were collected from a provincially inspected slaughterhouse. Specifically, broilers were electrically stunned, bled, eviscerated, and intestinal tracts (from ileum to cloaca) were collected in sterile plastic bags and transported on ice to a laboratory within 3 h. Cecal tissues were subsequently dissected in an anaerobic chamber (Bactron 300, Sheldon Manufacturing Incorporated; gas condition: 5% CO_2, 5% H_2, and 90% N_2) to collect cecal contents as described above.

Cecal samples from additional chickens were collected in LCY supplemented with 30% glycerol and 0.05% L-cysteine for culturing and isolating bacteria. Samples were obtained from 2-year-old backyard bantam rosters (n = 2), 17-week-old roosters from heritage breeds raised

without antibiotics and with access to outdoors (n = 5), 1-, 5- and 40-week-old layers raised in an organic system with access to outdoors (n = 5 per age), and 40-week-old layers raised in cages in an intensive system (n = 5). These birds were euthanized on farm using cervical dislocation, and the cecal contents were transported on dry ice and stored at -80°C until use.

3.5.2. DNA extraction

The extraction of DNA from cecal contents, reagent control, and a gut microbial community standard (ZymoBIOMICS, ZymoResearch) was performed using QIAamp DNA stool mini kit (Qiagen Inc. US) according to manufacturer's instructions with the addition of a bead-beating step. Approximately 100 mg of cecal content was mixed with Inhibitex® buffer and 2.0 mm garnet beads (BioSpec Products, Bartlesville, OK) and lysed by bead-beating twice at 6.0 m/s for 30 s (FastPrep-24TM 5G, MP Biomedicals). Purity and concentration of the extracted DNA were assessed using a Nanodrop™ 2000 spectrophotometer (Thermo Scientific™) and Quant-iT™ PicoGreen dsDNA assay kit (Thermo Scientific™). The Illumina 16S Metagenomic Sequencing Library Preparation protocol targeting the V3-V4 region of the 16S rRNA gene (primers forward 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; and reverse: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC). Each 25 µl of PCR reaction contained 12.5 µl of 2x KAPA HiFi HotStart ReadyMix, 5 µL of 1 µM forward primer, 5 µl of 1 µM reverse primer, and 2.5 µl of DNA template (5ng/µl). The PCR program consisted of an initial denaturation step of 3 min at 95°C, followed by 25 cycles of 95°C for 30s, 55°C for 30s, 72°C for 30s, and a final extension step of 72°C for 5 min. Amplicons were

purified using AMPure XP beads prior to and after attachment of Illumina sequencing adapters. The final library was diluted to 4nM and sequenced using paired-end 2 x 300 cycles on an Illumina MiSeq Platform (Illumina Inc, San Diego, CA). All DNA extractions and sequencing procedures were performed by the same person.

3.5.3. 16S rRNA amplicon sequencing analysis

Raw sequencing data were processed using Quantitative Insight into Microbial Ecology 2(QIIME 2 v2021.4) [78] and DADA2 for pairing, denoising, de-replication and chimera filtering [79]. Sequences were truncated at 270 (forward) and 220 (reverse) base pairs based on median quality score, and discarded if presented more than 6 expected errors. Mafft and fastree methods [80, 81] were used to align sequences and generate phylogenetic trees. Taxonomy was assigned using the q-2-feature-classifier plugin [82] Naïve Bayes classifier [83] pretrained on SILVA 138 QIIME compatible database [84]. Sequences were clustered at 99% identity using majority taxonomy strings. Data were analyzed using phyloseq v.1.40.0 [85], microbiome v. 1.18.0 [86] and qiime2R v. 0.99.6 [87] packages in R v.1.4.1717 [88]. Amplicon sequence variants (ASVs) assigned to Mitochondria family, Chloroplast order, Archaea kingdom or unassigned were removed from the dataset, and the remaining reads were rarefied at an even count for downstream analysis. Phylogenetic diversity, Chao1, and Simpson indices were used to evaluate alpha diversity. Bray-Curtis distance matrix and principal coordinates analysis (PCoA) were used to evaluate beta diversity. Hierarchical clustering was performed based on Bray-Curtis distance matrix and single linkage (“friends-of-friends”) method (stats package). Testing for differentially abundant features was performed at ASV, taxa, family and phylum levels. Analysis at the taxa level was performed by merging all ASVs exhibiting the same taxonomy string using *tax_glom*

function (phyloseq package). Differential abundance analyses were done using limma-voom tool v.3.52.4 [89, 90], DESeq2 (v.1.36.0) with apeglm for logarithmic fold change shrinkage and FDR correction [91, 92], and non-parametric factorial Kruskal-Wallis sum-rank test from linear discriminant analysis effect size (LEfSe) algorithm [93]. To reduce the occurrence of type I error, features were considered as differentially abundant if differences were consistently detected by a combination of the three methods, with alpha level of 0.05. The core microbiota for each system was defined as taxa present in at least 50% of birds in each group [94]. Taxa concomitantly assigned as core in the microbiota of IPS and EPS broilers were defined as members of core cecal microbiota. Figures were generated using using ggplot2 v.3.4.0 [95] and pheatmap v.1.0.12 [96] packages.

Phylogenetic investigation of communities by reconstruction of Unobserved States (PICRUSt2, v.2.1.4-b [97]) was used to predict functional potential of the microbiota based on ASVs. Sequences were aligned to reference trees using EPA-ng [98] and hidden state predictions were performed by castor package v. 1.7.8 [99]. Enzyme Commission numbers and MetaCyc pathways databases were used to predict microbial gene families and pathways.

3.5.7. Bacterial isolation and identification

Cecal samples collected in LCY supplemented with 30% glycerol and 0.05% L-cysteine were thawed on ice, homogenized by vortexing, and serial diluted on 1X PBS inside an anaerobic chamber. Diluted samples were plated on nine types of media (Table S1) and incubated at 37°C using three gas conditions: aerobic (AE), anaerobic (AN) (5% CO_2, 5% H_2, and 90% N_2), and high CO_2 (H) (20% CO_2, 10% H_2, and 70% N_2). After 72 h of incubation, single colonies with distinct

morphologies were selected from each plate, streaked on fresh media plates, and incubated for 72 h to obtain isolates. Purified colonies were further characterized by Sanger sequencing. Amplicon polymerase chain reaction (PCR) amplifying 16s rRNA gene was performed using primers 8F/926R (forward 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse: 5'-CCGTCAATTCNTTTRAGT-3'). Each 50 µl of PCR reaction contained 5 µL of 10x Taq polymerase buffer (Invitrogen, Carlsbad, CA), 2 µL of 10 µM forward primer, 2 µL of 10 µM reverse primer, 2 µL of 10 mM deoxynucleotide triphosphate mix (Invitrogen), 2 µL of 50 mM $MgCl_2$ (Invitrogen), 0.5 µL of 1 U/µL Taq polymerase (Invitrogen), and 1 µL of nuclease-free water containing the harvested bacterial colony. The PCR program consisted of an initial denaturation step of 10 min at 94°C, followed by 40 cycles of 94°C for 30s, 56°C for 30s, 72°C for 1 min, and a final extension step of 72°C for 7 min. The amplicon products were purified using a GeneJET Gel Extraction and DNA Cleanup Micro kit (Thermo Scientific). Purity and concentration of amplicon products were determined using Nanodrop 2000 (Thermo Scientific) and sent for Sanger sequencing (Molecular Biology Service Unit, University of Alberta). Taxonomy of the resultant sequences was analyzed using the rRNA/ITS database of 16S rRNA within the Basic Local Alignment Search Tool (BLAST) from National Center for Biotechnology Information (NCBI). The maximum likelihood phylogenetic tree based on 16S rRNA gene was constructed using MEGAX software and the phylogenetic tree was visualized using Interactive tree of life (iTOL v6) software. In addition, sterile 1 x PBS was added to each type of plate and the surface was scraped using an inoculation loop to collect microbial cells which were mixed with LCY supplemented with 0.05% L-cysteine and 50% glycerol (final concentration, 25%) and stored at -80°C.

3.5.8. Whole genome sequencing (WGS) analysis

The extraction of genomic DNA from selected isolates was conducted using the Wizard® Genomic DNA purification kit (Promega Corp. USA) following manufacturer's instructions. Purity and concentration of extracted DNA were determined using Nanodrop 2000 spectrophotometer and Quant-iT^{TM} PicoGreen dsDNA assay kit (Thermo Scientific™), respectively. The library preparation was performed using NEBNext® Ultra ™ II DNA Library Prep kit (New England Biolabs Inc., CA), followed by 150 bp paired-end sequencing on an Illumina NovaSeq 6000 platform (Illumina Inc., USA). The quality of resultant paired-end 150 bp sequences was analyzed using FastQC (v.0.11.9) [100] and sequence adapters were trimmed using Trimmomatic (v. 039) [101]. Draft genome assemblies were performed using SPAdes assembler v.30.10.1 [102] and the quality of assembled genomes were evaluated using QUAST v.5.5.0 [103]. For determination of taxonomy, the contig file of selected isolates was compared to reference genomes (RefSeq Genome database) of suspected species using OrthoANIu algorithm [104]. Isolates that concomitantly presented 16S rRNA identity below 98% against NCBI rRNA/ITS database using BLAST and OrthoANIu values lower than 95% against NCBI reference genomes were considered as new species [105]. Genome annotation was performed on Rapid Annotation using Subsystem Technology (RAST) system [106] and visualized using SEED Viewer [107]. Genemark [108] and BLASTp [109] were used to predict genes and align the resulting amino acid sequences of coding genes. The amino acid sequences were aligned against the Bacteriocin database [110] for identification of genes encoding bacteriocins, and the Virulence Factor Database (VFDB) for identification of genes encoding virulence factors using identity thresholds of > 60% (Blastp). Additionally, the Comprehensive Antibiotic Resistance Database (CARD) [111] was used to annotate antibiotic resistance genes using default parameters.

3.5.9. Statistical analysis

Alpha-diversity indices were analyzed using one-way ANOVA and Tukey HSD test when data were normally distributed; and Kruskal-Wallis and Wilcoxon rank sum test with FDR adjustment if data were not normally distributed. Beta-diversity distance matrices were analyzed using permutational multivariate analysis of variance (PERMANOVA) to test for differences in the distances to centroids and dispersion of the groups; and permutation test for homogeneity of multivariate dispersions (PERMDISP) to test for homogeneity of dispersions between groups (vegan package). Differences in the gene abundance or MetaCyc pathways in extensive and intensive systems were identified using ANOVA-like differential expression (ALDEx) analysis (ALDEx2 package v.1.30.0 [112]. Alpha level of 0.05 was considered for all the analyses.

3.6. REFERENCES

1. Mead GC. Microbes of the Avian Cecum: Types Present and Substrates Utilized. Journal of Experimental Zoology.1989;252(S3):48-54.

2. Józefiak D, Rutkowski A, Martin SA. Carbohydrate fermentation in the avian ceca: A review. Animal Feed Science and Technology. 2004;113:1–15.

3. Apajalahti J, Vienola K. Interaction between chicken intestinal microbiota and protein digestion. Anim Feed Sci Technol. 2016;221:323–30.

4. Broom LJ, Kogut MH. The role of the gut microbiome in shaping the immune system of chickens. Veterinary Immunology and Immunopathology. 2018;204:44–51.

5. Meijerink N, Kers JG, Velkers FC, van Haarlem DA, Lamot DM, de Oliveira JE, et al. Early Life Inoculation With Adult-Derived Microbiota Accelerates Maturation of Intestinal Microbiota and Enhances NK Cell Activation in Broiler Chickens. Front Vet Sci. 2020;7.

6. Zenner C, Hitch TCA, Riedel T, Wortmann E, Tiede S, Buhl EM, et al. Early-Life Immune System Maturation in Chickens Using a Synthetic Community of Cultured Gut Bacteria. mSystems. 2021;6.

7. Rubio LA. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poult Sci. 2019;98:695–706.

8. Chintoan-Uta C, Wisedchanwet T, Glendinning L, Bremner A, Psifidi A, Vervelde L, et al. Role of Cecal Microbiota in the Differential Resistance of Inbred Chicken Lines to Colonization by Campylobacter jejuni. 2020. https://doi.org/10.1128/AEM.

9. Zaytsoff SJM, Montina T, Boras VF, Brassard J, Moote PE, Uwiera RRE, et al. Microbiota Transplantation in Day-Old Broiler Chickens Ameliorates Necrotic Enteritis via Modulation of the Intestinal Microbiota and Host Immune Responses. Pathogens. 2022;11.

10. Forder REA, Howarth GS, Tivey DR, Hughes RJ. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. Poult Sci. 2007;86:2396–403.

11. Oh PL, Benson AK, Peterson DA, Patil PB, Moriyama EN, Roos S, et al. Diversification of the gut symbiont lactobacillus reuteri as a result of host-driven evolution. ISME Journal. 2010;4:377–87.

12. Kubasova T, Seidlerova Z, Rychlik I. Ecological adaptations of gut microbiota members and their consequences for use as a new generation of probiotics. International Journal of Molecular Sciences. 2021;22.

13. Ju T, Kong JY, Stothard P, Willing BP. Defining the role of Parasutterella, a previously uncharacterized member of the core gut microbiota. ISME Journal. 2019;13:1520–34.

14. Zilber-Rosenberg I, Rosenberg E. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. FEMS Microbiology Reviews. 2008;32:723–35.

15. Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? Trends in Ecology and Evolution. 2016;31:689–99.

16. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. Nature. 2017;548:43–51.

17. Blaser MJ, Falkow S. What are the consequences of the disappearing human microbiota? Nat Rev Microbiol. 2009;7:887–94.

18. Langella P, Guarner F, Martín R. Editorial: Next-generation probiotics: From commensal bacteria to novel drugs and food supplements. Frontiers in Microbiology. 2019;10 AUG.

19. Jha R, Das R, Oak S, Mishra P. Probiotics (Direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Animals. 2020;10:1–19.

20. Schneitz C. Competitive exclusion in poultry - 30 years of research. Food Control. 2005;16 8 SPEC. ISS.:657–67.

21. Stanley D, Hughes RJ, Geier MS, Moore RJ. Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: Challenges presented for the identification of performance enhancing probiotic bacteria. Front Microbiol. 2016;7 FEB.

22. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, et al. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell. 2017;171:1015-1028.e13.

23. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, et al. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science (1979). 2019;365.

24. Souza MR, Moreira JL, Barbosa FHF, Cerqueira MMOP, Nunes ÁC, Nicoli JR. Influence of intensive and extensive breeding on lactic acid bacteria isolated from Gallus gallus domesticus ceca. Vet Microbiol. 2007;120:142–50.

25. Seidlerova Z, Kubasova T, Faldynova M, Crhanova M, Karasova D, Babak V, et al. Environmental impact on differential composition of gut microbiota in indoor chickens in commercial production and outdoor, backyard chickens. Microorganisms. 2020;8.

26. Scupham AJ, Patton TG, Bent E, Bayles DO. Comparison of the cecal microbiota of domestic and wild turkeys. Microb Ecol. 2008;56:322–31.

27. Ferrario C, Alessandri G, Mancabelli L, Gering E, Mangifesta M, Milani C, et al. Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses. Environ Microbiol. 2017;19:4771–83.

28. Ocejo M, Oporto B, Hurtado A. 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. Sci Rep. 2019;9.

29. Nurmi E, Rantala M. New aspects of salmonella infection in broiler production. Nature. 1973;241:210–1.

30. Xu Y, Yang H, Zhang L, Su Y, Shi D, Xiao H, et al. High-throughput sequencing technology to reveal the composition and function of cecal microbiota in Dagu chicken. BMC Microbiol. 2016;16:1–9.

31. Pandit RJ, Hinsu AT, Patel N V., Koringa PG, Jakhesara SJ, Thakkar JR, et al. Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16s rDNA amplicon sequencing. Microbiome. 2018;6.

32. Paul SS, Chatterjee RN, Raju MVLN, Prakash B, Rao SVR, Yadav SP, et al. Gut microbial composition differs extensively among indian native chicken breeds originated in different geographical locations and a commercial broiler line, but breed-specific, as well as across-breed core microbiomes, are found. Microorganisms. 2021;9:1–22.

33. Bindari YR, Gerber PF. Centennial Review: Factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance. Poultry Science. 2022;101.

34. Das B, Ghosh TS, Kedia S, Rampal R, Saxena S, Bag S, et al. Analysis of the gut microbiome of rural and urban healthy indians living in sea level and high altitude areas. Sci Rep. 2018;8.

35. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107:14691–6.

36. Bensch HM, Tolf C, Waldenström J, Lundin D, Zöttl M. Bacteroidetes to Firmicutes: captivity changes the gut microbiota composition and diversity in a social subterranean rodent. Anim Microbiome. 2023;5.

37. Marcolla CS, Alvarado CS, Willing BP. Early life microbial exposure shapes subsequent animal health. Canadian Journal of Animal Science. 2019;99:661–77.

38. Karasova D, Faldynova M, Matiasovicova J, Sebkova A, Crhanova M, Kubasova T, et al. Host Species Adaptation of Obligate Gut Anaerobes Is Dependent on Their Environmental Survival. Microorganisms. 2022;10.

39. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Gut anaerobes capable of chicken caecum colonisation. Microorganisms. 2019;7.

40. Ramírez GA, Richardson E, Clark J, Keshri J, Drechsler Y, Berrang ME, et al. Broiler chickens and early life programming: Microbiome transplant-induced cecal community dynamics and phenotypic effects. PLoS One. 2020;15 11 November.

41. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One. 2019;14.

42. Rychlik I. Composition and function of chicken gut microbiota. Animals. 2020;10.

43. Farkas V, Csitári G, Menyhárt L, Such N, Pál L, Husvéth F, et al. Microbiota Composition of Mucosa and Interactions between the Microbes of the Different Gut Segments Could Be a Factor to Modulate the Growth Rate of Broiler Chickens. Animals. 2022;12.

44. Johnson TJ, Youmans BP, Noll S, Cardona C, Evans NP, Peter Karnezos T, et al. A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl Environ Microbiol. 2018;84.

45. Videnska P, Sedlar K, Lukac M, Faldynova M, Gerzova L, Cejkova D, et al. Succession and replacement of bacterial populations in the caecum of egg laying hens over their whole life. PLoS One. 2014;9.

46. Jurburg SD, Brouwer MSM, Ceccarelli D, van der Goot J, Jansman AJM, Bossers A. Patterns of community assembly in the developing chicken microbiome reveal rapid primary succession. Microbiologyopen. 2019;8.

47. Ngunjiri JM, Taylor KJM, Abundo MC, Jang H, Elaish M, Mahesh KC, et al. Farm stage, bird age, and body site dominantly affect the quantity, taxonomic composition, and dynamics of respiratory and gut microbiota of commercial layer chickens. Appl Environ Microbiol. 2019;85.

48. Kollarcikova M, Faldynova M, Matiasovicova J, Jahodarova E, Kubasova T, Seidlerova Z, et al. Different bacteroides species colonise human and chicken intestinal tract. Microorganisms. 2020;8:1–14.

49. Yan C, Xiao J, Li Z, Liu H, Zhao X, Liu J, et al. Exogenous Fecal Microbial Transplantation Alters Fearfulness, Intestinal Morphology, and Gut Microbiota in Broilers. Front Vet Sci. 2021;8.

50. Fan Y, Ju T, Bhardwaj T, Korver DR, Willing BP. Week-Old Chicks with High Bacteroides Abundance Have Increased Short-Chain Fatty Acids and Reduced Markers of Gut Inflammation . Microbiol Spectr. 2023. https://doi.org/10.1128/spectrum.03616-22.

51. Poudel B, Shterzer N, Sbehat Y, Ben-Porat N, Rakover M, Tovy-Sharon R, et al. Characterizing the chicken gut colonization ability of a diverse group of bacteria. Poult Sci. 2022;101.

52. Wongkuna S, Kumar R, Ghimire S, Mattiello Drescher S, Maji A, Ambat A, et al. Identification of a microbial sub-community from the feral chicken gut that reduces 1 Salmonella colonization and improves gut health in a gnotobiotic chicken model. 2. https://doi.org/10.1101/2022.07.23.501241.

53. Wienemann T, Schmitt-Wagner D, Meuser K, Segelbacher G, Schink B, Brune A, et al. The bacterial microbiota in the ceca of Capercaillie (Tetrao urogallus) differs between wild and captive birds. Syst Appl Microbiol. 2011;34:542–51.

54. Yadav S, Caliboso KD, Nanquil JE, Zhang J, Kae H, Neupane K, et al. Cecal microbiome profile of Hawaiian feral chickens and pasture-raised broiler (commercial) chickens determined using 16S rRNA amplicon sequencing. Poult Sci. 2021;100.

55. Barnes EC, Impby CS. The Occurence and Properties of Uric Acid Decomposing Anaerobic Bacteria in the Avian Caecum. Journal of Applied Bacteriology. 1974;37(3):393-409.

56. Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW, Pallen MJ. Extensive microbial and functional diversity within the chicken cecal microbiome. PLoS One. 2014;9.

57. Mancabelli L, Ferrario C, Milani C, Mangifesta M, Turroni F, Duranti S, et al. Insights into the biodiversity of the gut microbiota of broiler chickens. Environ Microbiol. 2016;18:4727–38.

58. Sueyoshi M, Adachi Y. Diarrhea Induced by Treponema hyodysenteriae: a Young Chick Cecal Model for Swine Dysentery. Infection and Immunity 1990;58(10):3348-3362.

59. Tokuda G, Mikaelyan A, Fukui C, Matsuura Y, Watanabe H, Fujishima M, et al. Fiber-associated spirochetes are major agents of hemicellulose degradation in the hindgut of wood-feeding higher termites. Proc Natl Acad Sci U S A. 2018;115:E11996–2004.

60. Borrelli L, Coretti L, Dipineto L, Bovera F, Menna F, Chiariotti L, et al. Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. Sci Rep. 2017;7.

61. Geissinger O, Herlemann DPR, Morschel E, Maier UG, Brune A. The ultramicrobacterium "Elusimicrobium minutum" gen. nov., sp. nov., the first cultivated representative of the termite group 1 phylum. Appl Environ Microbiol. 2009;75:2831–40.

62. Hou Q, Kwok LY, Zheng Y, Wang L, Guo Z, Zhang J, et al. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. Sci Rep. 2016;6.

63. Nan S, Yao M, Zhang X, Wang H, Li J, Niu J, et al. Fermented grape seed meal promotes broiler growth and reduces abdominal fat deposition through intestinal microorganisms. Front Microbiol. 2022;13.

64. Wang J, Fan H, Han Y, Wei J, Zhao J, Zhou Z. Pyrosequencing of the broiler chicken gastrointestinal tract reveals the regional similarity and dissimilarity of microbial community. Can J Anim Sci. 2017;97:302–13.

65. Yang Q, Ren S, Niu T, Guo Y, Qi S, Han X, et al. Distribution of antibiotic-resistant bacteria in chicken manure and manure-fertilized vegetables. Environmental Science and Pollution Research. 2014;21:1231–41.

66. Leong LEX, Khan S, Davis CK, Denman SE, McSweeney CS. Fluoroacetate in plants - a review of its distribution, toxicity to livestock and microbial detoxification. Journal of Animal Science and Biotechnology. 2017;8.

67. Zgheib R, Anani H, Meng MM, Mailhe M, Ricaboni D, Morand A, et al. New human-associated species of the family atopobiaceae and proposal to reclassify members of the genus olsenella. Int J Syst Evol Microbiol. 2021;71.

68. Colombino E, Biasato I, Ferrocino I, Oddon SB, Caimi C, Gariglio M, et al. Effect of insect live larvae as environmental enrichment on poultry gut health: Gut mucin composition, microbiota and local immune response evaluation. Animals. 2021;11.

69. Dame-Korevaar A, Kers JG, van der Goot J, Velkers FC, Ceccarelli D, Mevius DJ, et al. Competitive Exclusion Prevents Colonization and Compartmentalization Reduces Transmission of ESBL-Producing Escherichia coli in Broilers. Front Microbiol. 2020;11.

70. Polansky O, Sekelova Z, Faldynova M, Sebkova A, Sisak F, Rychlik I. Important metabolic pathways and biological processes expressed by chicken cecal microbiota. Appl Environ Microbiol. 2016;82:1569–76.

71. Siegerstetter SC, Schmitz-Esser S, Magowan E, Wetzels SU, Zebeli Q, Lawlor PG, et al. Intestinal microbiota profiles associated with low and high residual feed intake in chickens across two geographical locations. PLoS One. 2017;12.

72. Lundberg R, Scharch C, Sandvang D. The link between broiler flock heterogeneity and cecal microbiome composition. Anim Microbiome. 2021;3.

73. Pedroso AA, Lee MD, Maurer JJ. Strength Lies in Diversity: How Community Diversity Limits Salmonella Abundance in the Chicken Intestine. Front Microbiol. 2021;12.

74. van Kuijk SJA, Han Y, Garcia-Ruiz AI, Rodiles A. Hydroxychloride trace minerals have a positive effect on growth performance, carcass quality and impact ileal and cecal microbiota in broiler chickens. J Anim Sci Biotechnol. 2021;12.

75. Kabir SML. The role of probiotics in the poultry industry. International Journal of Molecular Sciences. 2009;10:3531–46.

76. Geirnaert A, Steyaert A, Eeckhaut V, Debruyne B, Arends JBA, Van Immerseel F, et al. Butyricicoccus pullicaecorum, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and small intestine conditions. Anaerobe. 2014;30:70–4.

77. Eeckhaut V, Wang J, Van Parys A, Haesebrouck F, Joossens M, Falony G, et al. The probiotic butyricicoccus pullicaecorum reduces feed conversion and protects from potentially harmful intestinal microorganisms and necrotic enteritis in broilers. Front Microbiol. 2016;7 SEP.

78. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.

79. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.

80. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.

81. Price MN, Dehal PS, Arkin AP. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5.

82. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018;6.

83. Pedregosa F, Michel V, Grisel O, Blondel M, Prettenhofer P, Weiss R, et al. Scikit-learn: Machine Learning in Python. 2011.

84. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 2013;41.

85. McMurdie PJ, Holmes S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One. 2013;8.

86. Lahti L, Shetty S. Introduction to the microbiome R package.

87. Bisanz JE. qiime2R: Importing QIIME2 artifacts and associated data into R sessions.

88. RStudio Team. RStudio: Integrated Development for R. 2020.

89. Law CW, Chen Y, Shi W, Smyth GK. METHOD Open Access voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 2014;3(15):R29.

90. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47.

91. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15.

92. Zhu A, Ibrahim JG, Love MI. Heavy-Tailed prior distributions for sequence count data: Removing the noise and preserving large differences. Bioinformatics. 2019;35:2084–92.

93. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12.

94. Martínez I, Stegen JC, Maldonado-Gómez MX, Eren MA, Siba PM, Greenhill AR, et al. The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes. Cell Rep. 2015;11:527–38.

95. Wickham H. ggplot2 Elegant Graphics for Data Analysis Second Edition. 2016.

96. Kolde Raivo. pheatmap: Pretty Heatmaps. 2019.

97. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38:685–8.

98. Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, et al. EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences. Systematic Biology. 2019;68:365–9.

99. Louca S, Doebeli M. Efficient comparative phylogenetics on large trees. Bioinformatics. 2018;34:1053–5.

100. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data . 2010.

101. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.

102. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology. 2012;19:455–77.

103. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: Quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5.

104. Yoon SH, Ha S min, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology. 2017;110:1281–6.

105. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018;9.

106. Aziz RK, Bartels D, Best A, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: Rapid annotations using subsystems technology. BMC Genomics. 2008;9.

107. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014;42.

108. Besemer J, Borodovsky M. GeneMark: Web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res. 2005;33 SUPPL. 2.

109. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic Local Alignment Search Tool. J Mol Biol. 1990;215:403–10.

110. van Heel AJ, de Jong A, Montalbán-López M, Kok J, Kuipers OP. BAGEL3: Automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides. Nucleic Acids Res. 2013;41

111. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res. 2020;48:D517–25.

112. Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. ANOVA-Like Differential Expression (ALDEx) Analysis for Mixed Population RNA-Seq. PLoS One. 2013;8.

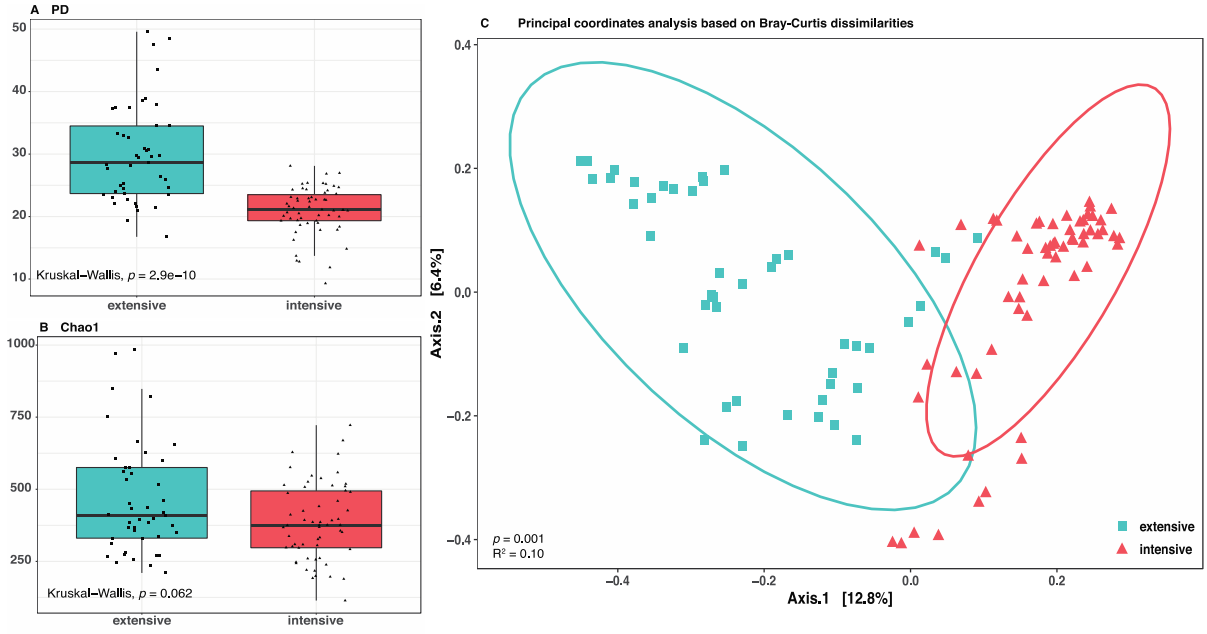


Figure 3.1. (A, B) Alpha-diversity indices and (**C**) principal coordinates analysis (PCoA) generated based on Bray-Curtis dissimilarity of cecal samples obtained from 35-day-old broilers from extensive or intensive production systems. Samples are colored and shaped according to treatments and data ellipses represent the 95% confidence region for group clusters assuming a multivariate t-distribution.

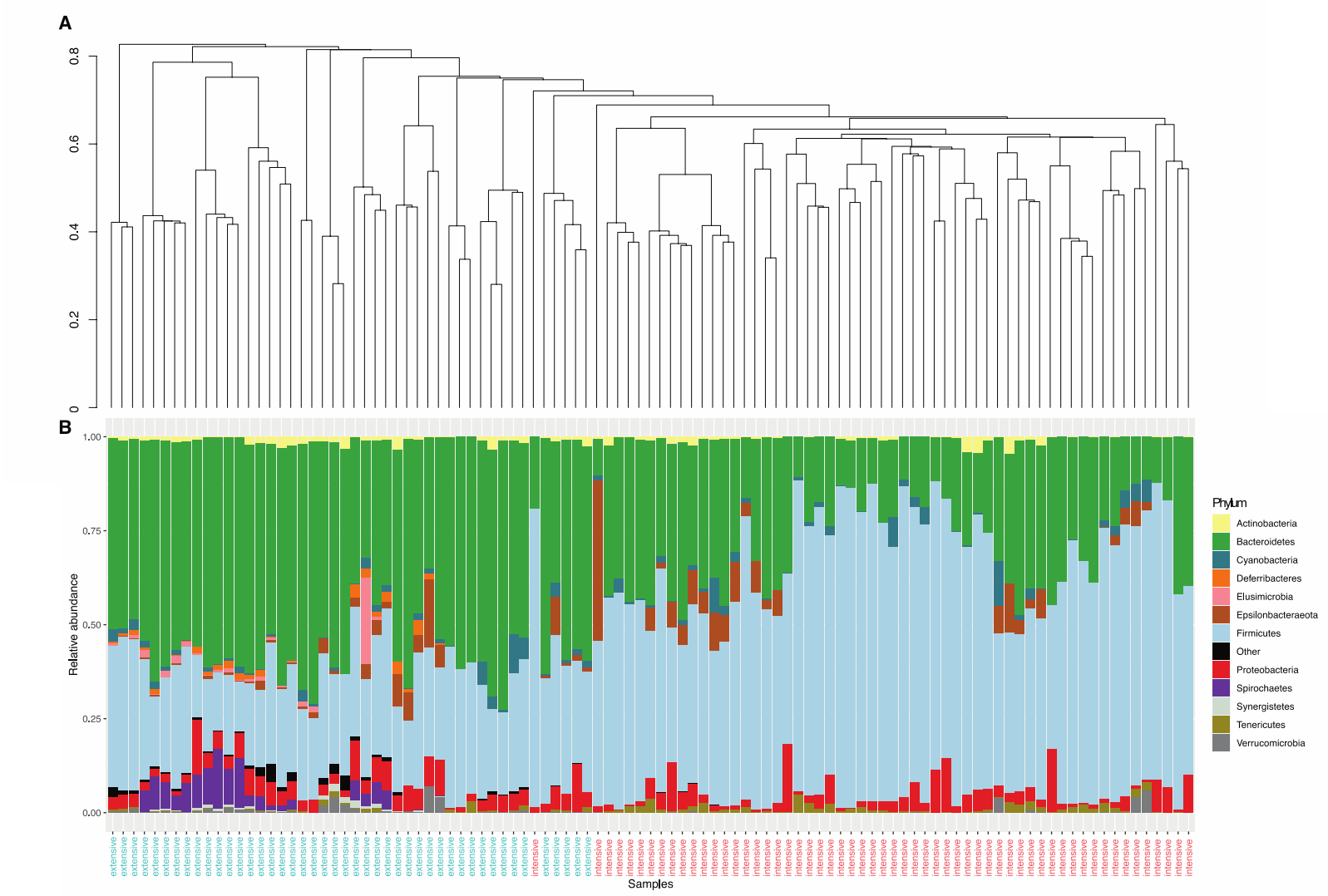

A
0.8
0.6
0.4
0.2
0
B
1.00
0.75
0.50
0.25
0.00
Relative abundance
Samples
Phylum
Actinobacteria
Bacteroidetes
Cyanobacteria
Deferribacteres
Elusimicrobia
Epsilonbacteraeota
Firmicutes
Other
Proteobacteria
Spirochaetes
Synergistetes
Tenericutes
Verrucomicrobia

Figure 3.2. (A) Dendrogram showing hierarchical clustering and (B) Bar plots showing the relative abundance of phyla in cecal samples obtained from 35-day-old broilers from extensive or intensive production systems. Phyla observed in less than 5% of samples and that had less than 1% relative abundance were combined as "Other" (black)**.**

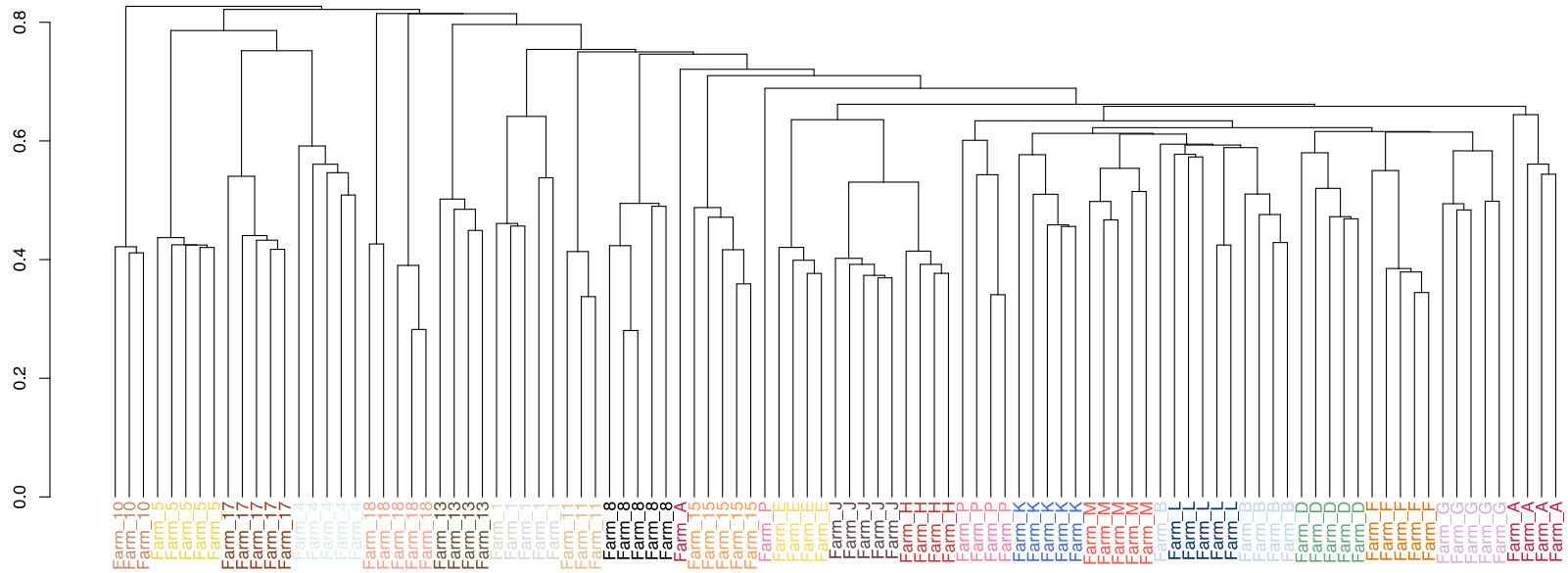


Figure 3. 3. Dendrogram showing hierarchical clustering of cecal samples from 35-day old broilers according to farm sources. Samples obtained from the same farm share the same color and identification. Identification of farms using extensive rearing practices are coded as "Farm_" followed by a number; whereas identification of farms using intensive rearing practices are coded as "Farm_" followed by a letter.

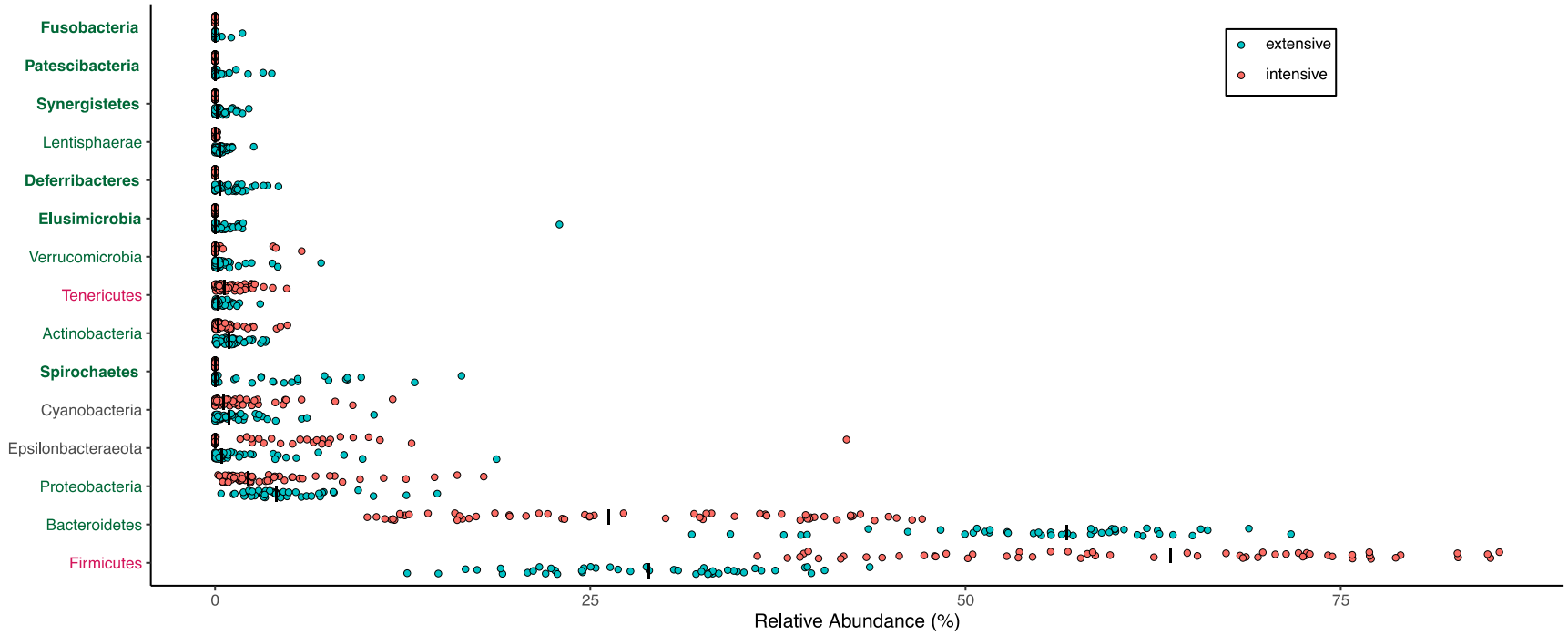


Figure 3. 4. The relative abundance of bacterial phyla in the cecal microbiota of 35-day-old broilers in extensive (green) and intensive (red) rearing systems. Names in green and red indicate phyla that are more abundant in broilers in extensive and intensive rearing systems, respectively. Names in gray indicate no differences in phyla abundance between systems. Bold indicates phyla that are exclusively present in the microbiota of broilers from extensive systems.

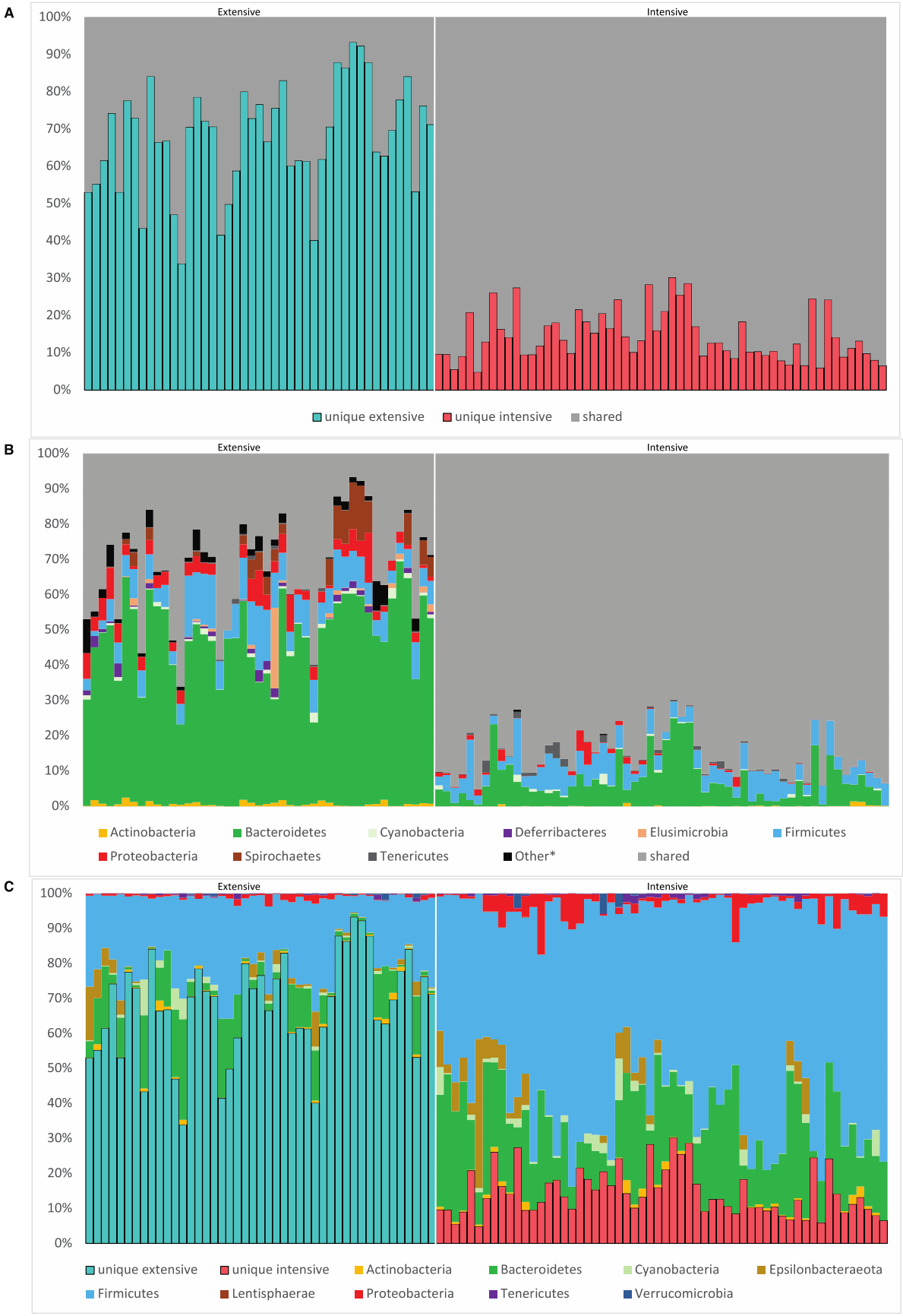

A
Extensive
Intensive
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
unique extensive
unique intensive
shared
B
Extensive
Intensive
Actinobacteria
Bacteroidetes
Cyanobacteria
Deferribacteres
Elusimicrobia
Firmicutes
Proteobacteria
Spirochaetes
Tenericutes
Other*
shared
C
Extensive
Intensive
unique extensive
unique intensive
Actinobacteria
Bacteroidetes
Cyanobacteria
Epsilonbacteraeota
Firmicutes
Lentisphaerae
Proteobacteria
Tenericutes
Verrucomicrobia

Figure 3. 5. A) The relative abundance of ASVs that are unique or shared in the cecal microbiota of 35-day-old broilers in extensive or intensive rearing systems, and the taxonomy at phylum-level of ASVs **B)** unique to each system and **C)** shared between the microbiota of birds in both systems.

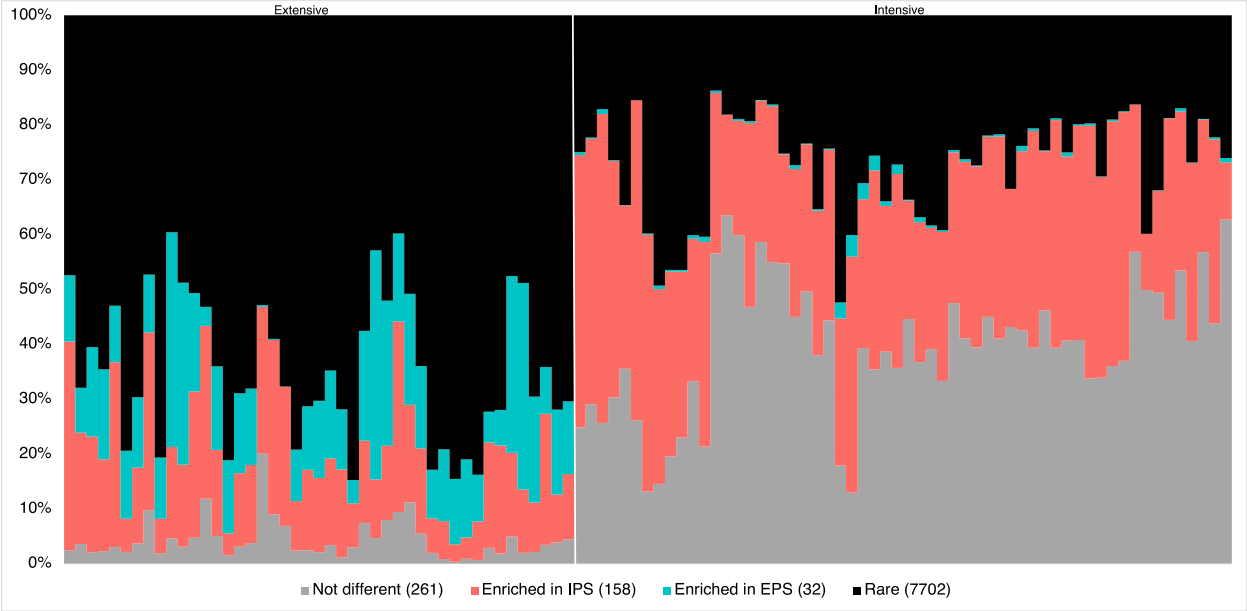


Figure 3.6. Simplified taxa plots showing the relative abundance of ASVs in cecal contents of 35-day-old broilers from extensive or intensive production systems. ASVs that were found to be enriched in the microbiota of extensively or intensively raised birds are shown in green and red, respectively. ASVs that were not differently abundant between the systems are shown in grey. ASVs observed in less than 20 birds were aggregated into a single category, called "Rare" and their relative abundance is indicated in black. The number between brackets indicated how many ASVs were included in each grouping category.

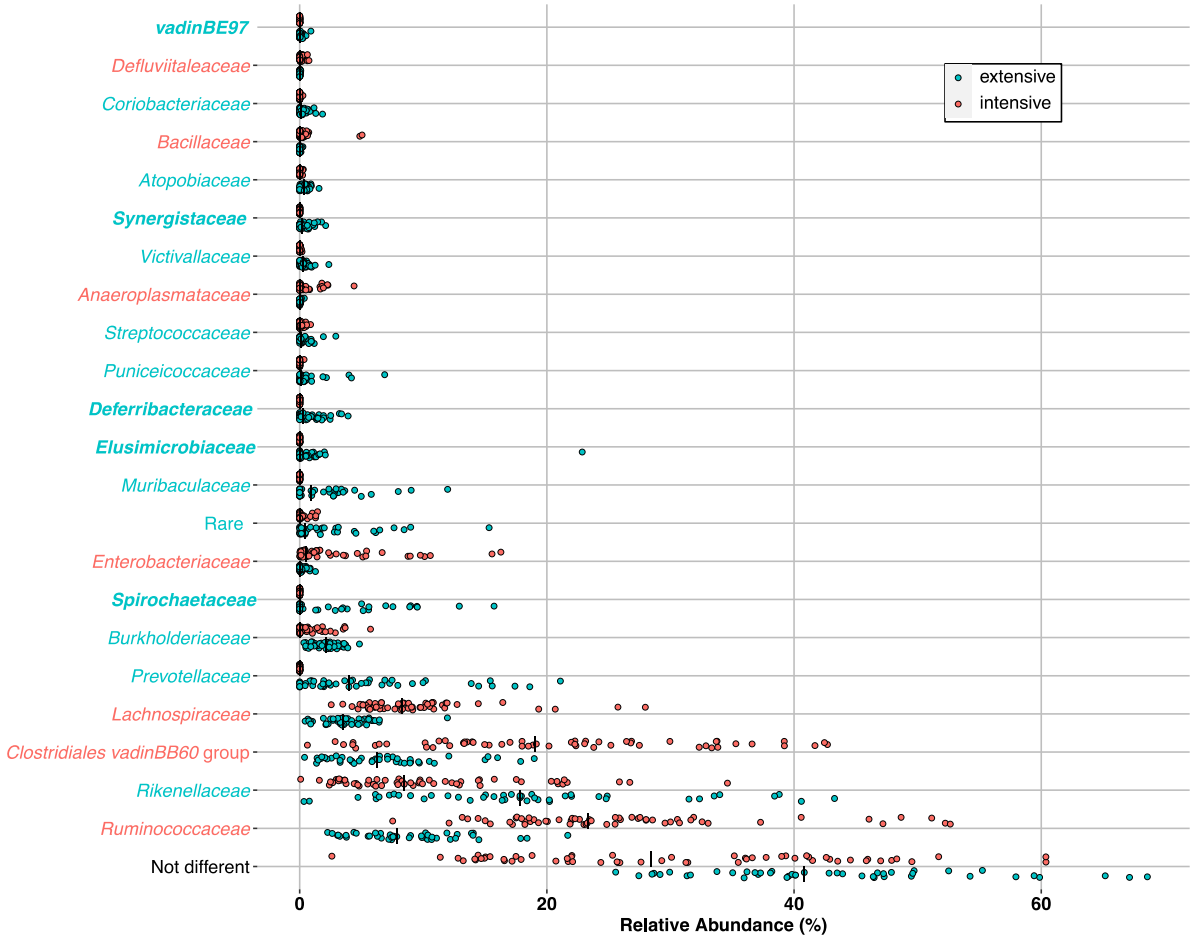


Figure 3.7. The relative abundance of bacteria families in the cecal microbiota of 35-day-old broilers in extensive (green) and intensive (red) rearing systems. Names in green and red indicate families that are more abundant in broilers in extensive and intensive rearing systems, respectively. Bold indicates families that are exclusively present in the microbiota of broilers from extensive systems. The "not different" indicates the relative abundance of bacterial families that were not differently abundant between the systems.

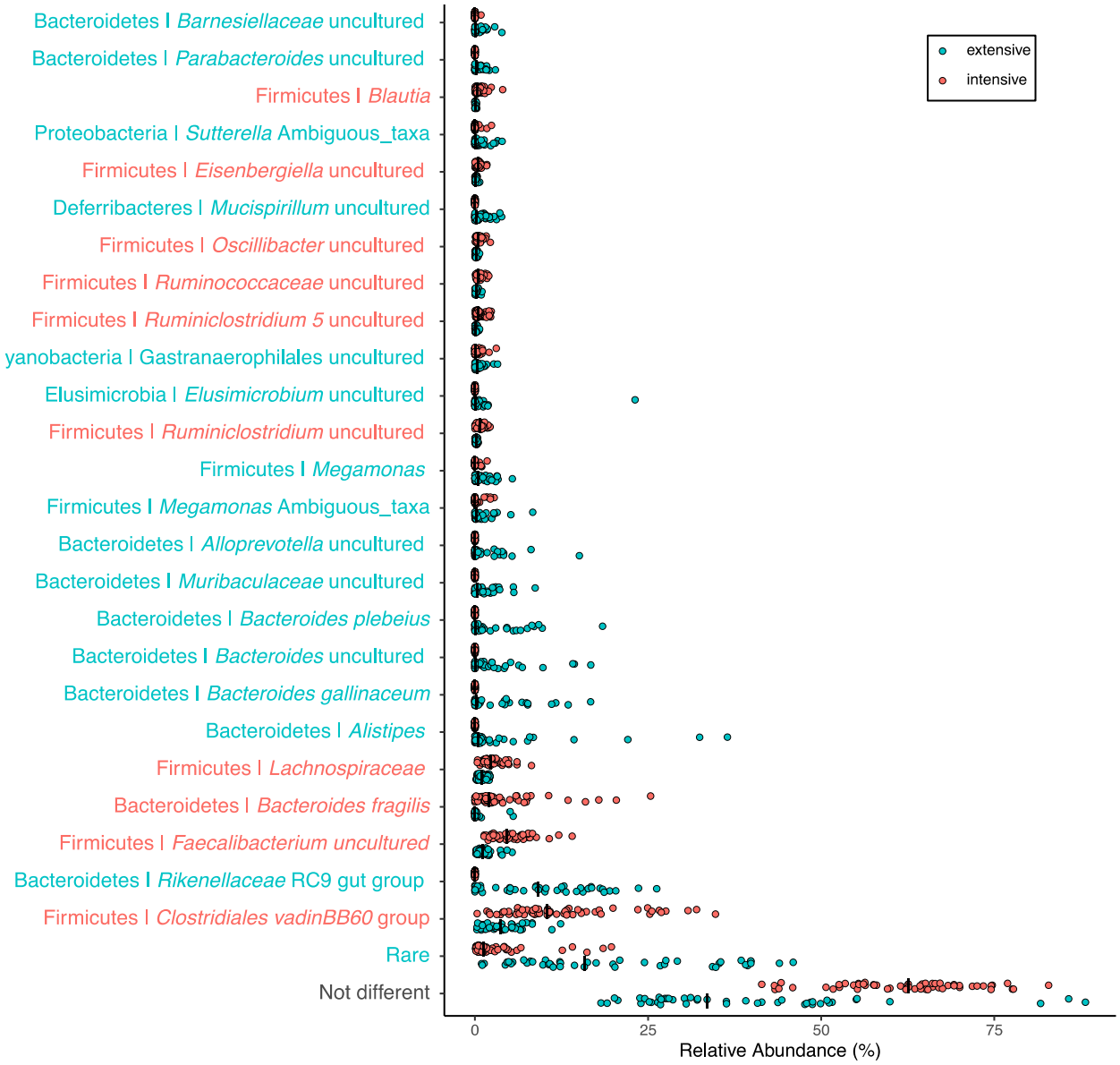


Figure 3.8. The relative abundance of microbial taxa that were shown to be differently abundant in the cecal microbiota of 35-day-old broilers from extensive or intensive production systems. Taxa names are color-coded according to the system in which they were found to be enriched: green for extensive system and red for intensive system. Dots represent the relative abundance of taxa in individual samples. Taxa observed in less than 20 birds were combined into a single category, called "Rare" and shown to be significantly enriched in EPS broilers. The relative

abundance of taxa that were not differently abundant between the systems is shown as “Not different”.

Figure 3.9. The relative abundance of bacteria taxa with relative abundance lower than 0.5% in the cecal microbiota of 35-day-old broilers in extensive (green) and intensive (red) rearing systems. Names in green and red indicate taxa that are more abundant in broilers in extensive and intensive rearing systems, respectively.

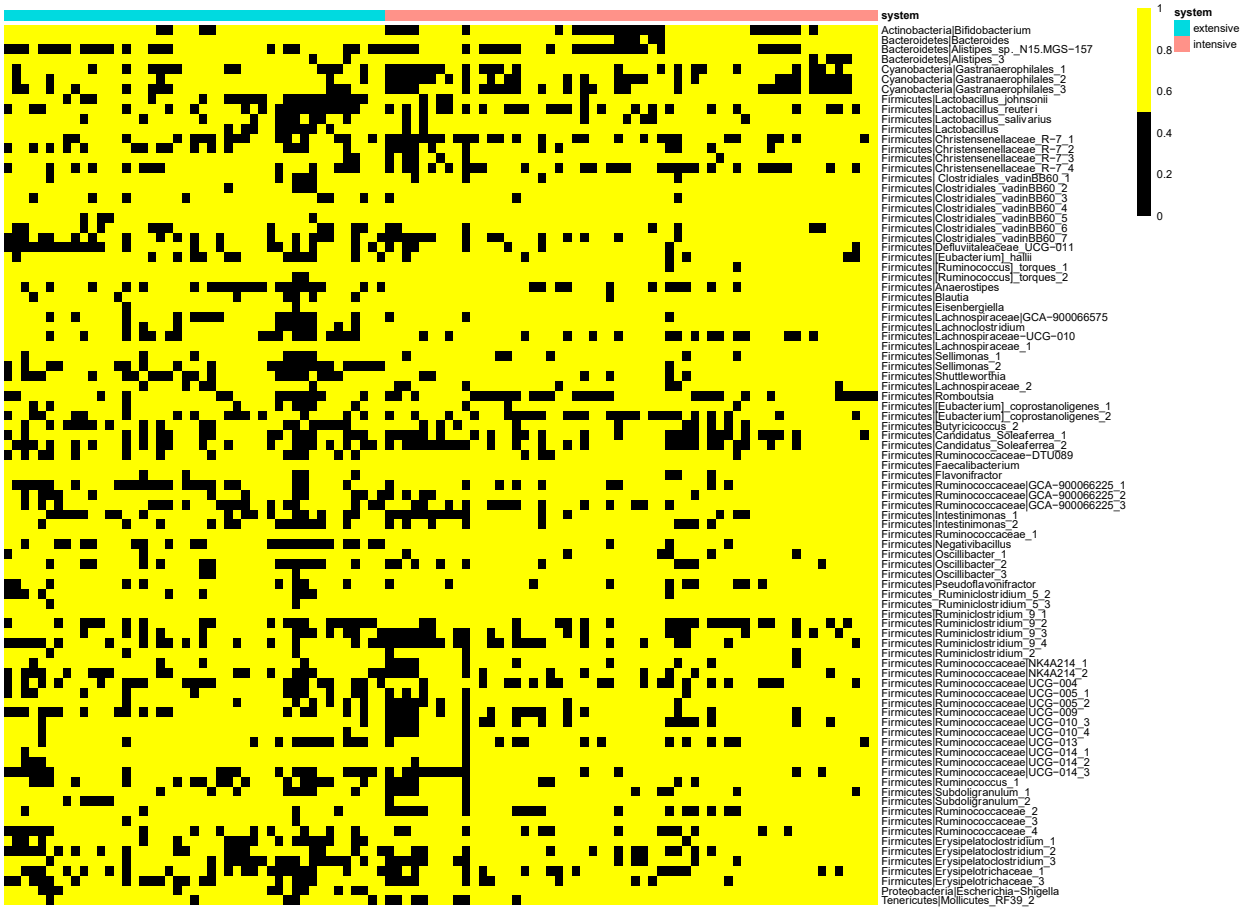


Figure 3.10. Heatmap indicating the presence/absence of taxa that were found to be core within the microbiota of 35-day-old broilers reared in extensive and intensive systems. Each column represents a sample and is colored in green (left side) or red (right side) if obtained from EPS or IPS broilers, respectively.

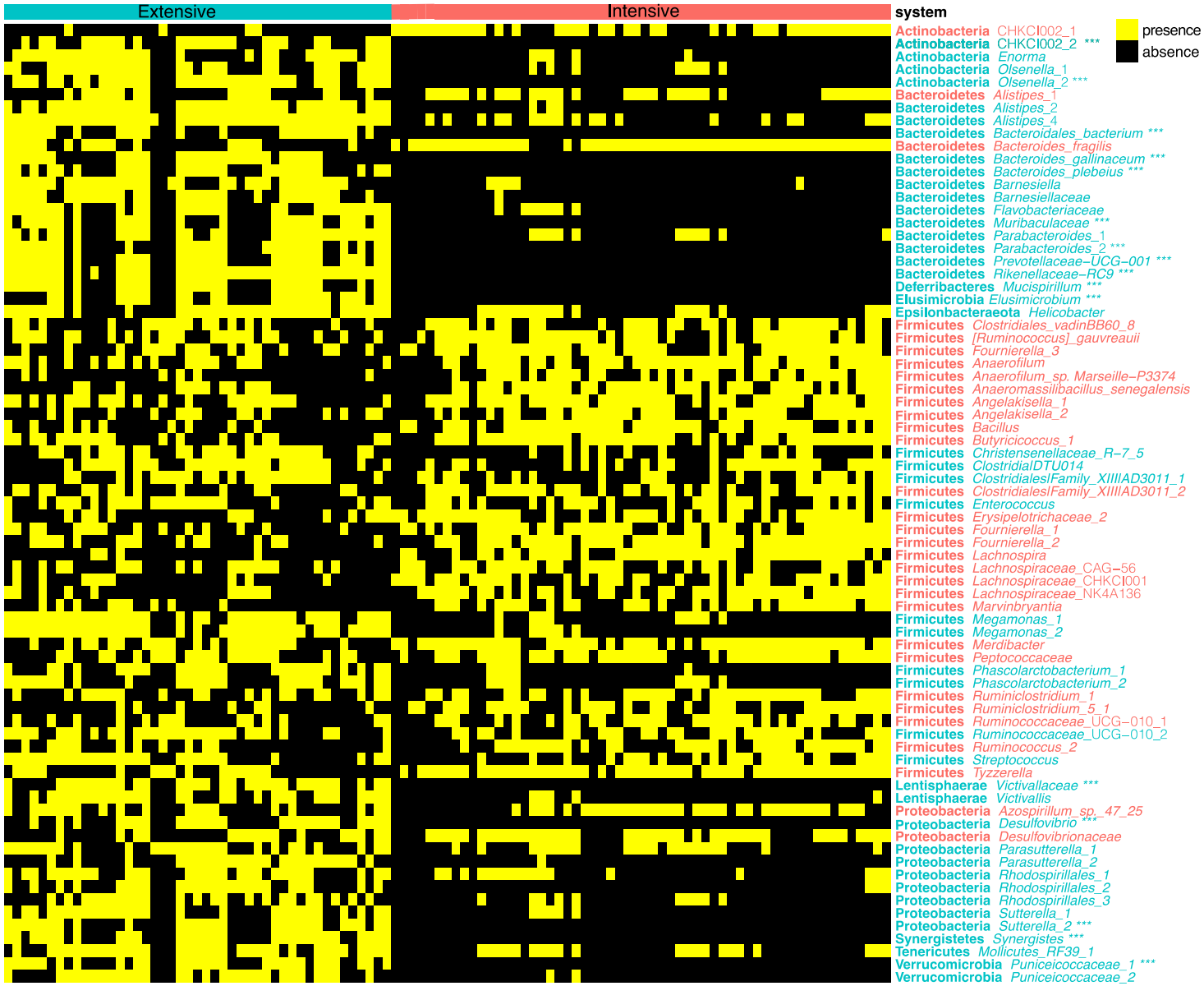

Extensive
Intensive
system
presence
absence
Actinobacteria CHKCI002_1
Actinobacteria CHKCI002_2 ***
Actinobacteria Enorma
Actinobacteria Olsenella_1
Actinobacteria Olsenella_2 ***
Bacteroidetes Alistipes_1
Bacteroidetes Alistipes_2
Bacteroidetes Alistipes_4
Bacteroidetes Bacteroidales_bacterium ***
Bacteroidetes Bacteroides_fragilis
Bacteroidetes Bacteroides_gallinaceum ***
Bacteroidetes Bacteroides_plebeius ***
Bacteroidetes Barnesiella
Bacteroidetes Barnesiellaceae
Bacteroidetes Flavobacteriaceae
Bacteroidetes Muribaculaceae ***
Bacteroidetes Parabacteroides_1
Bacteroidetes Parabacteroides_2 ***
Bacteroidetes Prevotellaceae_UCG_001 ***
Bacteroidetes Rikenellaceae_RC9 ***
Deferribacteres Mucispirillum ***
Elusimicrobia Elusimicrobium ***
Epsilonbacteraeota Helicobacter
Firmicutes Clostridiales_vadinBB60_8
Firmicutes [Ruminococcus]_gauvreauii
Firmicutes Fournierella_3
Firmicutes Anaerofilum
Firmicutes Anaerofilum_sp. Marseille_P3374
Firmicutes Anaeromassilibacillus_senegalensis
Firmicutes Angelakisella_1
Firmicutes Angelakisella_2
Firmicutes Bacillus
Firmicutes Butyricicoccus_1
Firmicutes Christensenellaceae_R_7_5
Firmicutes ClostridialDTU014
Firmicutes ClostridialesIFamily_XIIIIAD3011_1
Firmicutes ClostridialesIFamily_XIIIIAD3011_2
Firmicutes Enterococcus
Firmicutes Erysipelotrichaceae_2
Firmicutes Fournierella_1
Firmicutes Fournierella_2
Firmicutes Lachnospira
Firmicutes Lachnospiraceae_CAG_56
Firmicutes Lachnospiraceae_CHKCI001
Firmicutes Lachnospiraceae_NK4A136
Firmicutes Marvinbryantia
Firmicutes Megamonas_1
Firmicutes Megamonas_2
Firmicutes Merdibacter
Firmicutes Peptococcaceae
Firmicutes Phascolarctobacterium_1
Firmicutes Phascolarctobacterium_2
Firmicutes Ruminiclostridium_1
Firmicutes Ruminiclostridium_5_1
Firmicutes Ruminococcaceae_UCG_010_1
Firmicutes Ruminococcaceae_UCG_010_2
Firmicutes Ruminococcus_2
Firmicutes Streptococcus
Firmicutes Tyzzerella
Lentisphaerae Victivallaceae ***
Lentisphaerae Victivallis
Proteobacteria Azospirillum_sp._47_25
Proteobacteria Desulfovibrio ***
Proteobacteria Desulfovibrionaceae
Proteobacteria Parasutterella_1
Proteobacteria Parasutterella_2
Proteobacteria Rhodospirillales_1
Proteobacteria Rhodospirillales_2
Proteobacteria Rhodospirillales_3
Proteobacteria Sutterella_1
Proteobacteria Sutterella_2 ***
Synergistetes Synergistes ***
Tenericutes Mollicutes_RF39_1
Verrucomicrobia Puniceicoccaceae_1 ***
Verrucomicrobia Puniceicoccaceae_2

Figure 3.11. Heatmap indicating presence (yellow) and absence (black) of core microbes in the microbiota of IPS and EPS broilers. Each column represents a sample and is colored in green (left side) or red (right side) if obtained from EPS or IPS broilers, respectively. Taxa names are colored in green or red if found to be core taxa within the microbiota of EPS or IPS broilers, respectively.

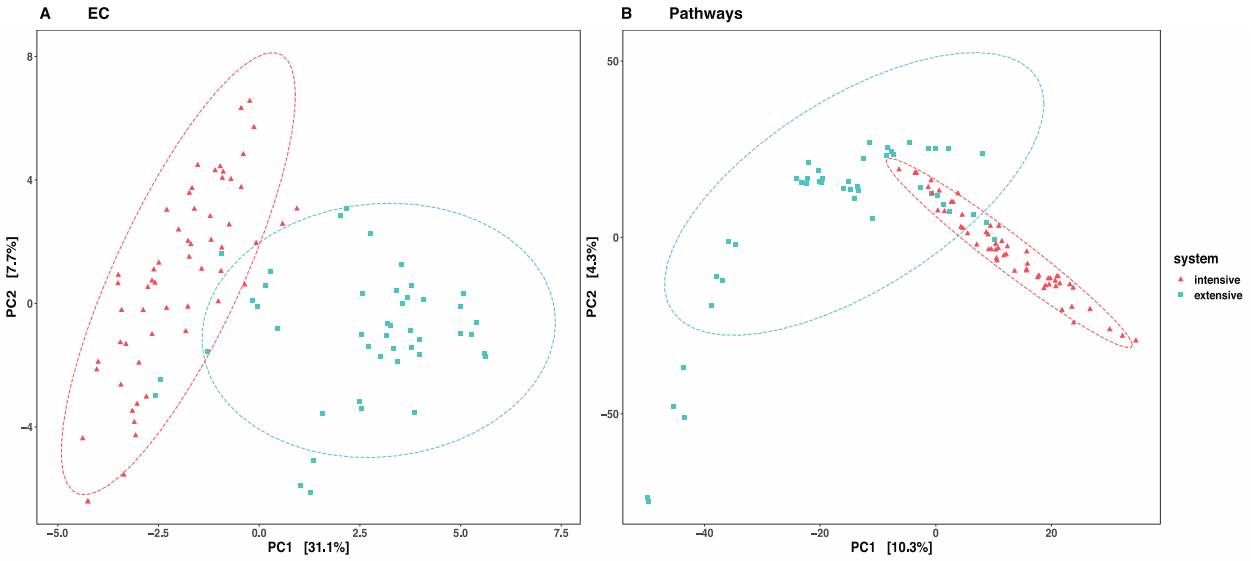


Figure 3.12. Principal component analysis of PICRUST2-predicted **A)** Enzyme Commission genes and **B)** Metacyc pathways in the cecal microbiota of 35-day-old broilers reared in intensive and extensive rearing systems.

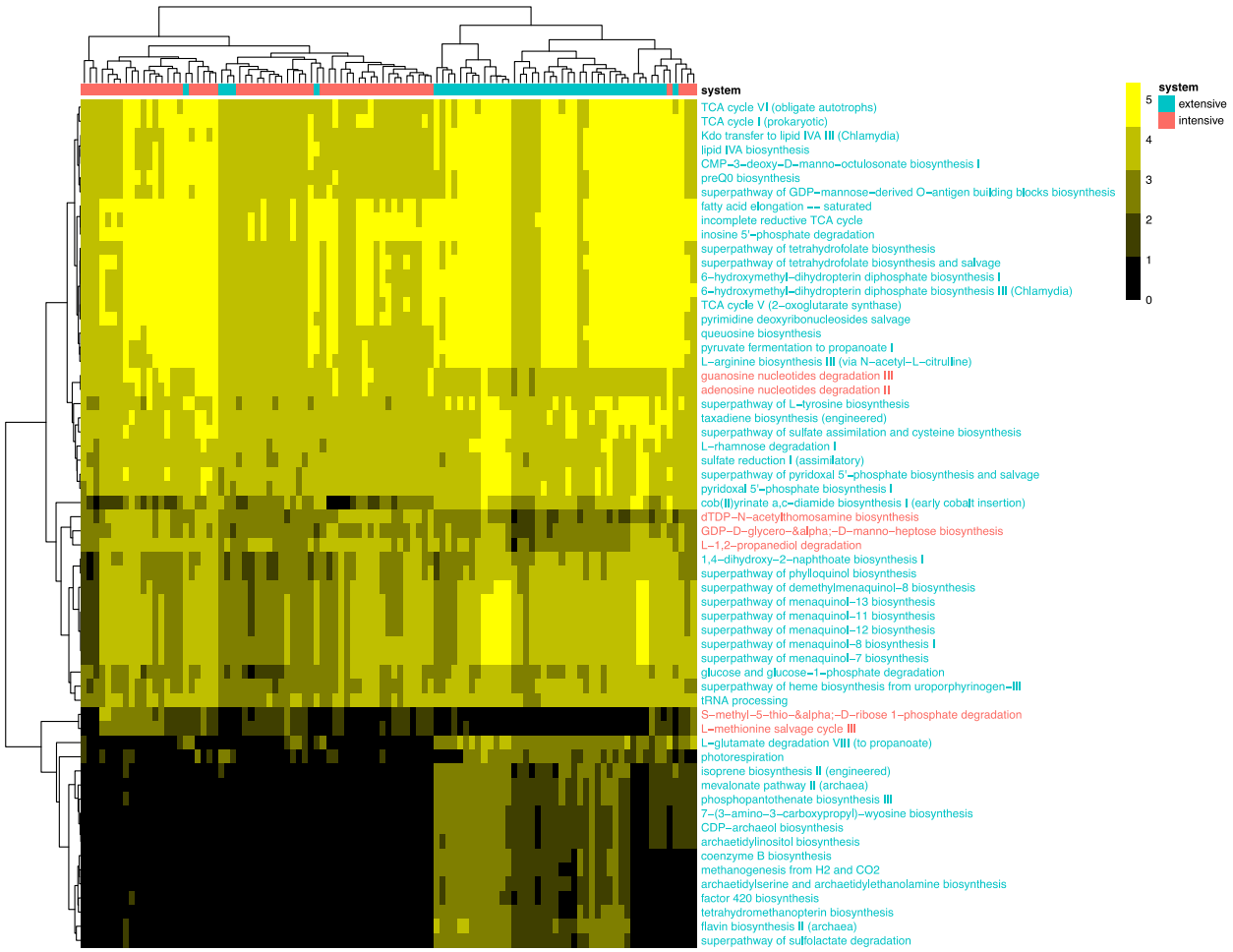


Figure 3.13. Heatmap indicating the log-transformed abundance of predicted pathways shown to be differently present in the microbiota of broilers from extensive (green) and intensive (red) rearing systems. Each column represents a sample and is colored in green or red if obtained from EPS or IPS broilers, respectively. Pathway names are colored in green or red if found to be enriched in the microbiota of EPS or IPS broilers, respectively.

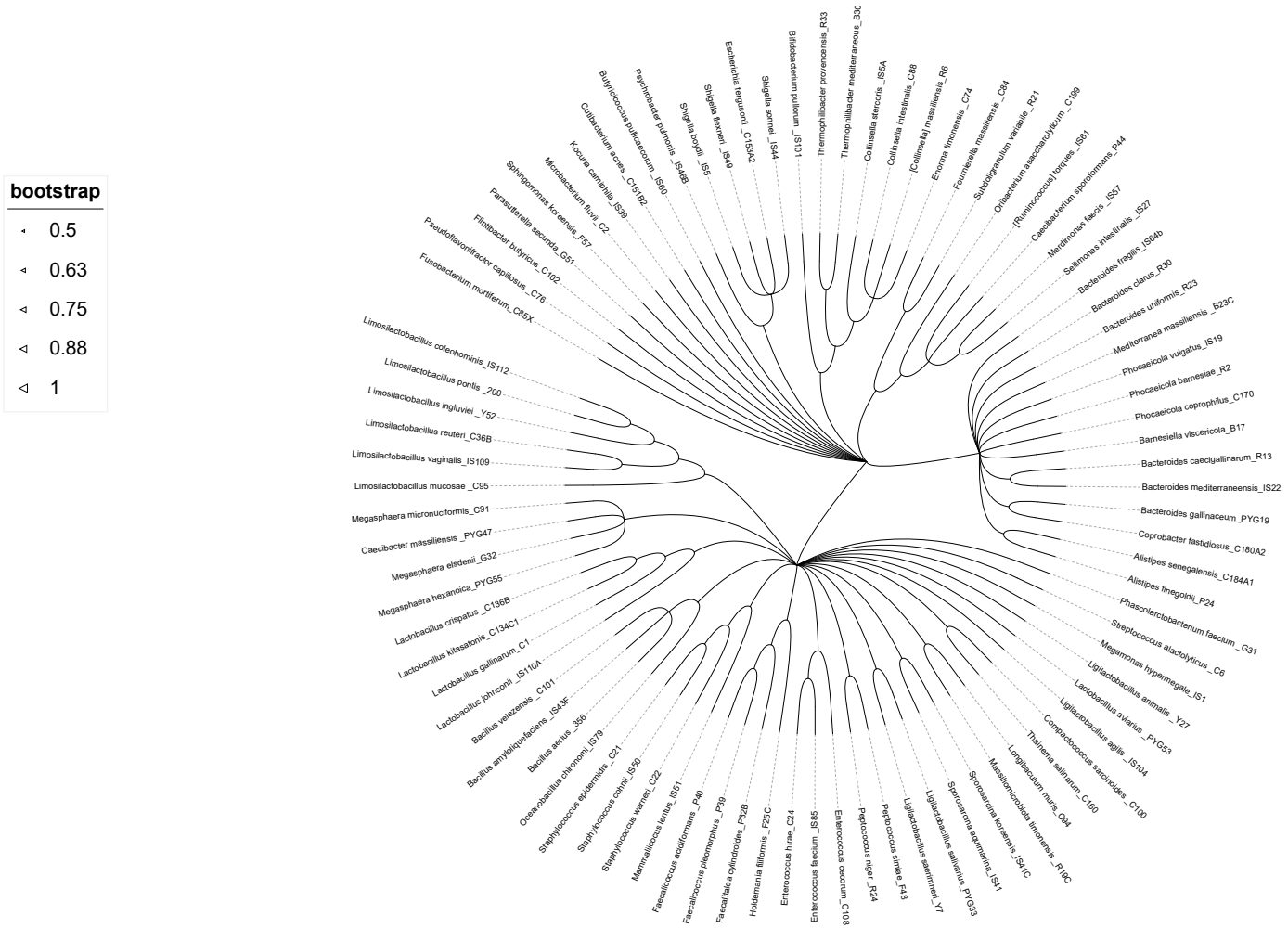

bootstrap
0.5
0.63
0.75
0.88
1
Blautia_obeum_ATCC_29174
Thermophilibacter provencensis_R33
Thermophilibacter mediterraneus_B30
Collinsella stercoris_IS5A
Collinsella intestinalis_C88
[Collinsella] massiliensis_R6
Enorma timonensis_C74
Fournierella massiliensis_C84
Subdoligranulum variabile_R21
Oribacterium sasacharolyticum_C169
[Ruminococcus] torques_IS61
Caecibacterium sporodormans_P44
Merdimonas faecis_IS57
Sellimonas intestinalis_IS27
Bacteroides fragilis_IS64b
Bacteroides clarus_R30
Bacteroides uniformis_R23
Mediterranea massiliensis_B23C
Phocaeicola vulgatus_IS19
Phocaeicola barnesiae_R2
Phocaeicola coprophilus_C170
Barnesiella viscericola_B17
Bacteroides caecigallinarum_R13
Bacteroides mediterraneensis_IS22
Bacteroides gallinaceum_PYG19
Coprobacter fastidiosus_C180A2
Alistipes senegalensis_C184A1
Alistipes finegoldii_P24
Phascolarctobacterium faecium_G31
Streptococcus alactolyticus_C6
Megamonas hypermegale_IS1
Ligilactobacillus animalis_Y27
Lactobacillus avarius_PYG53
Ligilactobacillus agilis_IS104
Companilactobacillus sanchicoles_C100
Thalnema salinarum_C160
Longibaculum muris_C84
Massilimicrobiota timonensis_R16C
Sporosarcina koreensis_IS41C
Sporosarcina aquimarina_IS41
Ligilactobacillus salivarius_PYG33
Ligilactobacillus saerimneri_Y7
Peptococcus simae_F48
Peptococcus niger_R24
Enterococcus cecorum_C108
Enterococcus faecium_IS85
Enterococcus hirae_C24
Holdemania filiformis_P25C
Faecalitalea cylindroides_P22B
Faecalicoccus pleomorphus_P39
Faecalicoccus acidiformans_P40
Mammaliicoccus lentus_IS51
Staphylococcus warneri_C22
Staphylococcus cohnii_IS56
Staphylococcus epidermidis_C21
Oceanobacillus chronomi_IS79
Bacillus aerius_356
Bacillus amyloliquefaciens_IS43F
Bacillus velezensis_C101
Lactobacillus johnsonii_IS110A
Lactobacillus gallinarum_C1
Lactobacillus kitasatonis_C134C1
Lactobacillus crispatus_C136B
Megasphaera hexanoica_PYG55
Megasphaera elsdenii_G32
Caecibacter massiliensis_PYG47
Megasphaera micronuciformis_C91
Limosilactobacillus mucosae_C95
Limosilactobacillus vaginalis_IS109
Limosilactobacillus reuteri_C36B
Limosilactobacillus ingluviei_Y52
Limosilactobacillus pontis_200
Limosilactobacillus coleohominis_IS112
Fusobacterium mortiferum_C85X
Pseudoflavonifractor capillosus_C76
Flintibacter butyricus_C102
Parasutterella secunda_G51
Sphingomonas koreensis_F57
Microbacterium fluvii_C2
Kocuria carniphila_IS39
Cutibacterium acnes_C151B2
Butyricicoccus pullicaecorum_IS60
Psychrobacter pulmonis_IS46B
Shigella boydii_IS5
Shigella flexneri_IS49
Escherichia fergusonii_C153A2
Shigella sonnei_IS44

Figure 3.14. Phylogram showing the diversity of the 87 bacterial species isolated from the cecal microbiota of chickens. Letters and numbers after the species name indicate the identification number of representative isolate for each species. The color in the circle represents the phyla: Actinobacteria (salmon), Bacteroidetes (yellow), Cyanobacteria (green), Firmicutes (blue), Fusobacteria (purple) and Proteobacteria (pink)

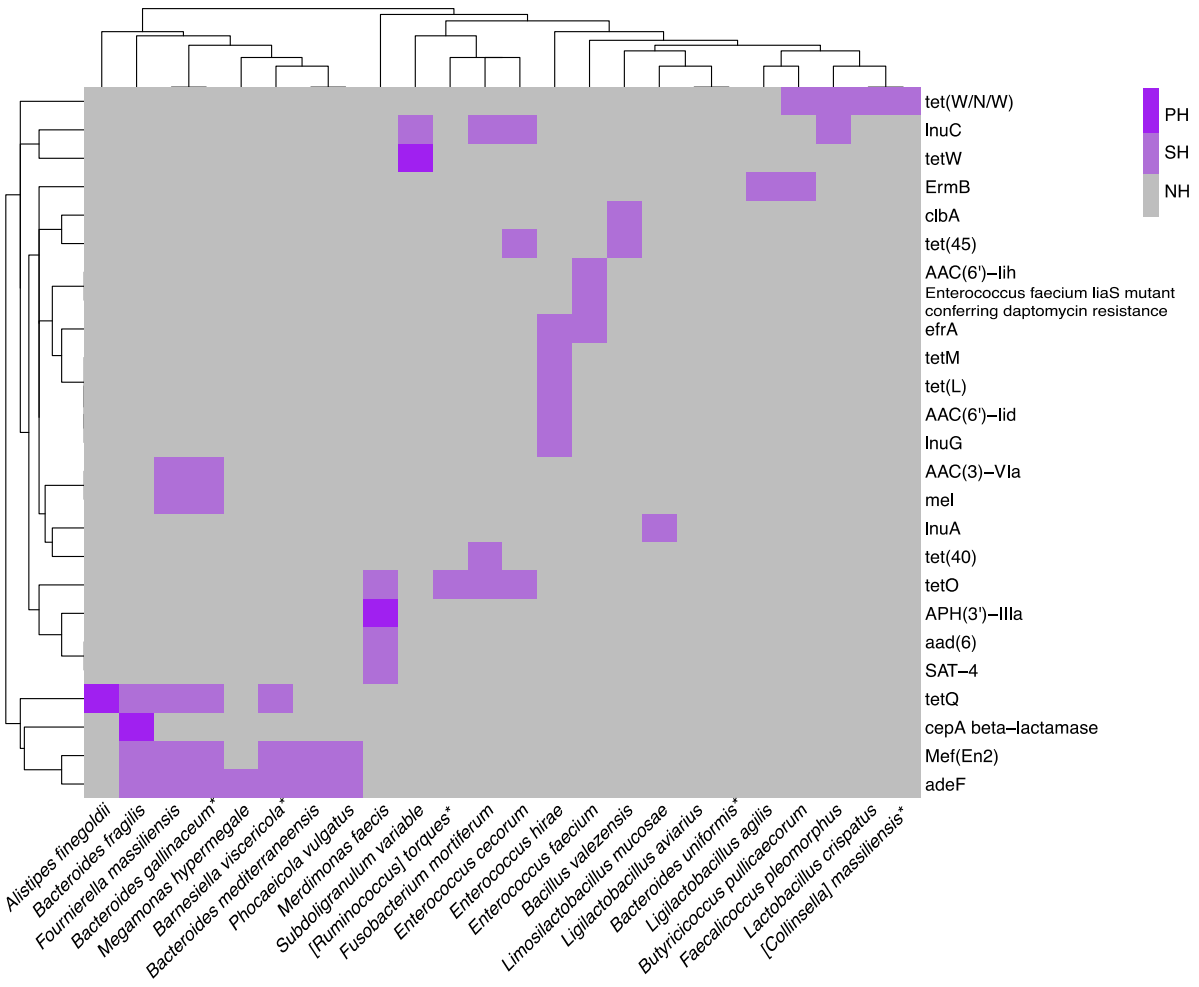


Figure 3.15. Heatmap indicating the presence of genes conferring antimicrobial resistance in the genome of selected isolates. Dark purple color indicates an RGI match 100% identical to the reference protein sequence (perfect hit, PH); the light purple color indicates an RGI match with a bit-score greater than the curated BLASTP bit-score cut-off (strict hit, SH), and the grey color indicates no RGI match (NH). Species names followed by * indicate isolates considered novel specie

Table 3.1. Summary of bacterial isolates. The table indicates the total number of bacterial isolates that were assigned to each species (n), the availability of whole genomes (WGS), the media in which bacteria were isolated from (media), the atmospheric conditions in which bacteria were incubated (atmosp.), the chicken source from which isolates were originated (source). The column BP indicates the range in the length of sequences obtained during Sanger sequencing. Columns QC, PID and Accession indicate the range of query cover and percentage identities and accession numbers according to NCBI rRNA/ITS database using BLAST. Definitions of media, atmosphere and source acronyms are provided at the footnote. Isolates considered to be novel species are indicated by *.

Species	n	WGS	media	atmosp.	source	BP	QC	PID	Accession
*[Collinsella] massiliensis**	15	YES	BHI, RCM, WC	AN, H	SP, HR	573-712	89-100	94-97	NR_144579.1
Bifidobacterium pullorum	1	NO	FAA	AN	NA	637	85	98	NR_029137.1
Collinsella intestinalis	2	NO	RCM, WC	H	SP, CL40	585-705	89-97	93-95	NR_113165.1
Collinsella stercoris	1	NO	RCM	H	NA	475	93	83	NR_113164.1
Cutibacterium acnes	2	NO	BHI	H	NA	509-729	82-98	93-98	NR_040847.1
Enorma timonensis	2	NO	FAA	H	BY	669-670	93-95	92-93	NR_144707.1
Kocuria carniphila	2	NO	BHI	AE	BY	709-753	98	98	NR_027193.1
Microbacterium fluvii	1	NO	BHI	AE	CL40	670	96	95	NR_041561.1
Thermophilibacter mediterraneous	12	NO	BHI, FAA, WC	AN, H	BY, SP, HR	482-730	74-99	92-94	NR_173691.1

Thermophilibacter provencensis	5	NO	BHI, RCM, WC	AN, H	SP, HR, CL40	669-724	79-99	92-98	NR_173690.1
Bacteroides mediterraneensis	1	YES	FAA	AN	BY	199	40	91	NR_144744.1
Alistipes finegoldii	2	YES	LSBA	AN	OB5	512-603	91	97-97	NR_115300.1
Alistipes senegalensis	3	NO	WC	AN	SP	668-737	88-98	95-95	NR_118219.1
Bacteroides caecigallinarum	3	NO	RCM	H	HR	679-771	98-99	94-96	NR_145844.1
Bacteroides clarus	1	NO	RCM	H	HR	768	94	90.09	NR_113065.1
Bacteroides fragilis	8	YES	FAA	AN	OB5, OL1, CB5	442-796	75-98	90-99	NR_074784.2
*Bacteroides gallinaceum**	2	YES	LSBA, PYG	AN	OB5, HR	539-701	88-92	84-95	NR_148822.1
*Bacteroides uniformis**	1	YES	RCM	H	HR	756	96	93.13	NR_112945.1
*Barnesiella viscericola**	1	YES	BHI	H	HR	777	99	96	NR_041508.1
Coprobacter fastidiosus	1	NO	WC	H	SP	780	99	99	NR_118316.1
Mediterranea massiliensis	2	NO	BHI	H	HR	662-737	94-98	97-98	NR_144747.1
Phocaeicola barnesiae	5	NO	RCM, WC	AN, H	NA	586-747	93-99	93-97	NR_041446.1
Phocaeicola coprophilus	1	NO	WC	H	SP	791	90	98.94	NR_041461.1
Phocaeicola vulgatus	2	YES	BHI	AN	CB5	758-784	98-99	98-99	NR_074515.1

Compactococcus sarcinoides	1	NO	BHI	AN	CL40	423	66	86	NR_176589.1
Thainema salinarum	3	NO	BHI, WC	H	SP, CL40	458-576	70-78	83-84	NR_176552.1
*[Ruminococcus] torques**	1	YES	FAA	AN	OL1	681	90	94.1	NR_036777.1
Bacillus aerius	1	NO	BHI	AE	BY	356	76	93	NR_118439.1
Bacillus amyloliquefaciens	2	NO	BHI	AE	BY	627-718	92-93	94-98	NR_117946.1
Bacillus velezensis	2	YES	BHI	AN	CL40	555-771	78-95	96-99	NR_116240.1/NR_075005.2
Butyricicoccus pullicaecorum	2	YES	FAA	AN	OL1, CL40	606-731	88-93	96-99	NR_044490.1
Caecibacter massiliensis	24	NO	PYG, WC	H	SP, HR	592-723	89-100	87-91	NR_147376.1
Caecibacterium sporoformans	1	NO	RCM	H	CL40	748	99	98	NR_159147.1
Enterococcus cecorum	3	YES	BHI, FAA, WC	H, AE	OL40, SP, CL40	546-780	86-98	94-99	NR_024905.1
Enterococcus faecium	9	YES	BHI, FAA, WC	AE	BY	402-795	78-98	89-99	NR_114742.1, NR_113904.1
Enterococcus hirae	1	YES	FAA	AE	OB5	796	97	99.08	NR_114783.2
Faecalicoccus acidiformans	1	NO	FAA, RCM, WC	AE	CL40	563	86	96	NR_134029.1

Faecalicoccus pleomorphus	11	YES	FAA, RCM, WC	AN	SP, CL40	284-790	85-100	82-99	NR_044660.3
Faecalitalea cylindroides	9	NO	FAA, RCM	AN, H	HR, CL40	695-771	97-100	98.59	NR_113163.1
Flintibacter butyricus	1	NO	BHI	AN	CL40	704	83	96	NR_144611.1
Fournierella massiliensis	4	YES	FAA, WC	AN	OL40, CL40	430-635	74-98	91-95	NR_156911.1
Holdemania filiformis	1	NO	FAA	H	HR	751	95	94	NR_029335.1
Lactobacillus aviarius	1	YES	PYG	H	HR	731	99	96.3	NR_112692.1
Lactobacillus crispatus	4	YES	MRS, WC	AN, H	OL1, OL5, SP, CL40	437-755	68-93	94-99	NR_119274.1
Lactobacillus gallinarum	3	NO	BHI, MRS, RCM	AN, H, AE	OL40, HR, CL40	740-802	91-95	97-99	NR_042111.1
Lactobacillus johnsonii	6	NO	BHI, MRS, RCM, YCFA	AN, H, AE	HR	768-800	88-99	98-99	NR_117574.1
Lactobacillus kitasatonis	2	NO	MRS	AN	OL5, OL40, SP	673-790	85-97	98-100	NR_024813.1
Ligilactobacillus agilis	12	YES	BHI, LSBA, RCM	AN, AE	BY, CL40	448-718	72-94	89-99	NR_113259.1
Ligilactobacillus animalis	3	NO	YCFA	H	HR	662-756	94-98	97-98	NR_041610.1

Ligilactobacillus saerimneri	1	NO	YCFA	H	HR	748	92	95	NR_029085.1
Ligilactobacillus salivarius	14	NO	BHI, MRS, PYG, YCFA, WC	AN, H, AE	BY, OL5, OL40, HR	439-780	71-99	92-99	NR_112759.1
Limosilactobacillus coleohominis	2	NO	MRS, YCFA	AN, H	HR	744-793	98	95-98	NR_042436.1
Limosilactobacillus ingluviei	2	NO	MRS, YCFA	H, AE	HR	581-756	90-95	93-99	NR_028810.1
Limosilactobacillus mucosae	1	YES	RCM	AN	CL40	701	99	95	NR_024994.1
Limosilactobacillus pontis	1	NO	YCFA	H	NA	656	97	94	NR_036788.2
Limosilactobacillus reuteri	6	NO	MRS, YCFA, WC	AN, H, AE	OB5, SP, HR, CL40	511-755	80-99	93-98	NR_075036.1
Limosilactobacillus vaginalis	6	NO	RCM, YCFA	AN, H	HR, CL40	718-789	96-99	96-99	NR_041796.1
Longibaculum muris	1	NO	RCM	AN	CL40	758	96	94	NR_144615.1
Mammaliicocus lentus	1	NO	FAA	AE	CB5	637	90	96	NR_043418.1
Massiliomicrobiota timonensis	5	NO	BHI, RCM	AN, H	BY, HR, CL40	569-747	83-99	92-97	NR_144738.1

Megamonas hypermegale	8	YES	BHI, FAA	H	BY	405-752	69-99	88-99	NR_025514.1
Megasphaera elsdenii	4	NO	GAM+V, RCM, PYG	AN, H	HR, CL40	650-704	85-100	89-91	NR_102980.1
Megasphaera hexanoica	16	NO	RCM, PYG	AN, H	HR, CL40	602-697	84-99	87-91	NR_157635.1
Megasphaera micronuciformis	4	NO	BHI, RCM, PYG	AN, H	HR, CL40	542-687	86-93	87-92	NR_025230.1
Merdimonas faecis	1	YES	FAA	AN	OL1	778	98	99	NR_157642.1
Oceanobacillus chironomi	1	NO	BHI	AE	NA	700	90	97	NR_043700.1
Oribacterium asaccharolyticum	1	NO	WC	AN	SP	566	82	88.87	NR_125571.1
Peptococcus niger	8	NO	BHI, FAA, RCM	H	HR	508-730	72-98	58-89	NR_113393.1
Peptococcus simiae	6	NO	FAA, RCM, YCFA	H	HR	484-716	95-97	83-88	NR_153710.1
Phascolarctobacterium faecium	54	NO	BHI, FAA, GAM+V	H	HR	698-774	75-99	88-93	NR_026111.1
Sellimonas intestinalis	1	NO	FAA	AN	BY	486	73	89	NR_148624.1
Sporosarcina aquimarina	2	NO	BHI	AE	BY	473-728	82-96	90-97	NR_025049.1
Sporosarcina koreensis	1	NO	BHI	AE	BY	695	91	97	NR_043526.1
Staphylococcus cohnii	1	NO	BHI	AE	CB5	705	86	98	NR_036902.1

Staphylococcus epidermidis	1	NO	FAA	AE	OB5	803	98	99	NR_113957.1
Staphylococcus warneri	1	NO	FAA	AE	OB5	799	98	99	NR_025922.1
Streptococcus alactolyticus	41	NO	BHI, FAA, RCM, PYG, WC	AN	OB5, OL40, SP	401-813	71-99	88-100	NR_041781.1
Subdoligranulum variabile	1	YES	RCM	H	HR	371	95	85.8	NR_028997.1
Pseudoflavonifractor capillosus	1	NO	FAA	AN	CL40	752	92	96	NR_025670.1
Fusobacterium mortiferum	2	YES	FAA	AN	CL40	722-728	84-85	98	NR_117734.1
Escherichia fergusonii	5	NO	FAA, WC	AN, AE	OB5, HR, CL40	434-753	70-99	92-98	NR_074902.1
Parasutterella secunda	8	NO	GAM+V, RCM	H	HR	714-776	98-100	97-99	NR_113328.1
Psychrobacter pulmonis	3	NO	BHI	AE	BY	400-744	71-98	90-99	NR_118026.1
Shigella boydii	1	NO	FAA	AN	NA	647	89	98	NR_104901.1
Shigella flexneri	1	NO	BHI	AE	CB5	696	98	96	NR_026331.1
Shigella sonnei	12	NO	BHI, FAA, WC	AN, AE	BY, OL40, SP,	512-766	73-95	93-100	NR_104826.1

					CL40, CB5				

Acronyms for media are defined as BHI: Blood heart infusion agar (Oxoid, CA); FAA: Fastideus anaerobe agar (Neogen, US); GAM+V: GAM agar modified "nissui" supplemented with vancomycin (Hyserve, Germany); LSBA: Lauryl sulfate broth agar media (Sigma, US); MRS: de Man, Rogosa, and Sharpe agar (BD Difco, US); PYG: Peptone yeast glucose media (DSMZ); RCM: reinforced clostridial agar (BD, US); WC:Wilkins-Chalgren anaerobe agar (Oxoid, CA); YCFA: Yeast casitone fatty acods (DSMZ). For atmosphere conditions: AE: aerobic; AN: Anaerobic: 5% CO2, 5% H2, and 90% N2; H: High CO2 (H) (20% CO2, 10% H2, and 70% N2). The chicken source originating the isolates are defined as BY: 2-year-old backyard bantam rosters; CB5: 5-week-old broilers raised in IPS; CL40:40-week-old layer raised in cages in IPS; HR:17-week-old roosters from heritage breeds raised without antibiotics and with access to outdoors; NA: origin not available; OB5:5-week-old broilers raised in an organic system; OL1: 1-week-old layers raised in an organic system; OL40: 40-week-old layers raised in an organic system; SP: EPS broilers collected from the provincially inspected slaughter plant.

Table 3.2. Summary of OrthoANI value results of whole genomes of isolates against reference genomes. Isolates considered to be novel species are indicated by *.

Taxonomy	Number of contigs	Average aligned length (bp)	OrthoANIu value (%)
*[Ruminococcus] torques**	185	801,623	74.03
Alistipes finegoldii	79	2,320,915	98.82
Bacillus valezensis	34	2,601,489	97.76
Bacteroides fragilis	62	3,433,269	99.07
*Bacteroides gallinaceum**	315	993,335	74.2
Bacteroides mediterraneensis	266	2,063,344	90.95
*Bacteroides uniformis**	280	1,207,048	75.03
Bacteroidetes/Phocaeicola vulgatus	123	3,767,576	99.99
*Barnesiella viscericola**	117	1,466,209	85.73
Butyricicoccus pullicaecorum	134	1,759,002	96.39
*[Collinsella] massiliensis**	39	549,191	75.93
Enterococcus cecorum	1,383	1,278,838	95.31
Enterococcus faecium	305	1,023,585	76.89
Enterococcus hirae	151	1,840,215	98.29
Faecalicoccus pleomorphus	86	1,232,468	98.17
Fournierella massiliensis	268	1,745,015	90.25

Fusobacterium mortiferum	1,977	1,141,297	84.47
Lactobacillus crispatus	93	1,219,605	98.12
Ligilactobacillus agilis	127	1,318,745	97.64
Ligilactobacillus aviarius	45	1,042,076	95.92
Limosilactobacillus mucosae	153	1,048,304	87.11
Megamonas hypermegale	260	1,375,194	98.29
Merdimonas faecis	146	1,918,807	97.81
Subdoligranulum variable	99	1,373,641	83.05

CHAPTER 4: Cecal microbiota development and physiological responses of broilers following early-life microbial inoculation using different delivery methods and microbial sources

4.1. INTRODUCTION

Intensive poultry production systems rely on several practices to minimize exposures to potential pathogens that could lead to increased mortality, disease outbreaks and contamination of food products. On broiler breeder farms, eggs are collected soon after being laid using automated systems to lower the chance of fecal contamination of eggshells. Fertilized eggs are incubated in sanitized hatcheries and chicks are born in an environment that is depleted of chicken commensal microbes compared to an environment where a hen is present. Newly hatched chicks are subsequently transported to empty barns and have limited opportunities to contact intestinal chicken microbiota unless reused/recycled litter is used (1). Throughout the production cycle, broilers are fed standardized diets comprising a limited number of ingredients that may also contain growth promoting antibiotics. Consequently, broilers raised in intensive farming systems may lack commensal microbes that they would encounter in natural environments (2, 3). On the contrary, wild and feral chicks are hatched in nests containing plant, feather and fecal material, stay close to the hen for at least 6 weeks of age, and forage for a variety of foods (4), thus favoring the transmission and natural selection of beneficial commensals across generations (5).

The chicken cecal microbiota contains Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria as the major phyla and their abundance is affected by numerous factors such as age, environment, genetics, and diet (6-10). It has been reported that broilers raised in intensive systems present a higher abundance of Firmicutes and a lower abundance of Bacteroidetes compared to chickens in free-range systems (2, 3). In addition, broilers from a fat genetic line had lower relative

abundance of Bacteroidetes compared to broilers from a lean genetic line (8). It has also been reported that the abundance of Proteobacteria was higher in young chicks compared to older birds (11,12). The development and maturation of the gut microbiota can exert significant influence on disease resistance (13) and growth performance (14), therefore microbial manipulation can potentially be used to improve animal health and performance.

This study aimed to investigate the cecal microbiota of broilers after early life exposure to bacteria harvested from different sources using three inoculation strategies. In the first experiment (inoculation strategies), we analyzed the microbiota of chicks exposed to cecal contents or microbial culture derived from cecal contents as an attempt to identify bacteria that can effectively colonize the broiler ceca, and to verify if exposure to microbial cultures exerts similar effects on the microbiota development as those promoted by exposure to cecal contents. We also evaluated the use of oral gavage and spraying the inoculum into the bedding as delivery methods. In the second experiment (cohousing), one bird from each cage received cecal contents by oral gavage to evaluate if microbes from the inoculated bird (seeder bird) would be effectively transmitted to its cage mate. In the third experiment (competition experiment), we tested the colonization capability of the cecal microbiota from extensively or intensively raised donors introduced to newly hatched broilers. Our objectives were 1) to evaluate if microbial cultures can mimic the composition of cecal contents and promote similar effects on microbiota community when introduced to chicks; 2) to identify bacteria that can consistently colonize and persist in the chicken gut environment after a single exposure, despite the composition of the inoculum being introduced; 3) to evaluate the effectiveness of delivery routes (oral gavage, spraying the inoculum into the bedding, and cohousing); and 4) to test whether the cecal microbiota obtained from extensively or intensively raised chickens is more capable to colonize the ceca of modern broilers.

4.2. MATERIALS AND METHODS

Experimental procedures were approved by the Institutional Animal Care and Use Committee, in accordance with the Canadian Council on Animal Care guidelines (AUP no. 00002572 and AUP no. 00002373).

4.2.1. Animals and housing

For all experiments, one-day old non-sexed broilers (Ross 708, Aviagen, Huntsville, AL) obtained from a commercial hatchery were neck-tagged and randomly distributed in sterile polycarbonate isolators with microfilter tops (Ancare corp., Bellmore, NY). Isolators contained autoclaved aspen shavings and were equipped with a feeder and a bell drinker. All experimental procedures were performed in a biosafety cabinet to avoid cross contamination between treatments and to reduce the influence of environmental factors that could confound results. Chicks were fed a commercial laboratory-grade antibiotic-free corn–soybean meal-based diet (Laboratory chick diet S-G 5065, Labdiet, St. Louis, MO) with *ad libitum* access to feed and water throughout the experiments. Isolators were kept in a temperature-controlled room with an 18-hour light, 6-hour dark daily lighting schedule. For the first three days of the experiments, the room temperature was kept at 30°C, and was gradually reduced as birds aged, reaching 24°C after three weeks.

4.2.2. Preparation of inocula

Donor chickens. In our study, broiler chicks were inoculated with microbiota obtained from chickens of different genetic lines and ages. Cecal samples used as inocula were selected from another study that analyzed cecal microbiota from 100 chickens in extensive and intensive

commercial farming systems. Samples were selected based on having above the average number of observed ASVs and distinct composition to provide a higher bacterial diversity when used as inocula. The composition of the inocula at the phylum level is indicated in Figure 4.1.A. The goal of the current study was to identify bacteria that are good colonizers of the chicken gut and we expected to identify specific bacteria that consistently colonize the inoculated chicks regardless of inoculum composition and source, and these bacteria would be of interest for future studies.

Cecal contents used in the first experiment (inoculation strategies) were obtained from a 40-week-old Lohmann white layer raised in battery cages from an intensive commercial operation. The bird was vaccinated against Marek's disease, Newcastle disease virus, avian encephalomyelitis, infectious bronchitis virus, *Eimeria*, and *Escherichia coli*, and did not receive any antibiotics in feed or water. In the second experiment (cohousing), cecal contents were obtained from a 40-week-old bantam rooster (eA) from a backyard flock. This sample was also used in the third experiment (competition experiment), along with cecal contents of a 35-day-old Cornish Cross Rock broiler (eB) raised in a free-range organic system. The birds eA and eB had access to the outdoors, did not receive any antibiotics in feed or water, and were chosen as representatives of gut microbiotas from extensive production systems. On the contrary, cecal contents collected from two 35-day-old broilers, one Ross 308 (iA) and one Ross 708 (iB), were used as representatives of gut microbiotas from intensive production systems. The birds iA and iB were vaccinated against Marek's disease virus and infectious bursal disease at the hatchery and received antibiotics and coccidiostats in feed (bacitracin, narasin, and nicarbazin) throughout the production cycle. The bird iA also received penicillin G and streptomycin in water for the first 5 days of life.

All donor chickens were euthanized on farm using cervical dislocation. Approximately 1 g of cecal contents were collected in sterile 1.5-ml tubes for 16S rRNA gene amplicon sequencing analysis and in sterile 5-ml microcentrifuge tubes containing 2.5 ml liquid casein yeast media (LCY) supplemented with 30% glycerol and 0.05% L-cysteine for bacterial culturing and for use as cecal content inocula. All samples were transported on dry ice and stored at -80°C.

Inocula preparation and handling. Cecal contents in LCY were thawed on ice in an anaerobic chamber (Bactron 300, Sheldon Manufacturing Incorporated; gas condition, 5% CO2, 5% H2, and 90% N2), homogenized, diluted in sterile LCY supplemented with 0.05% L-cysteine and 50% glycerol (final concentration, 25%), aliquoted in 5-ml tubes (1 tube per isolator) and stored at -80°C until inoculation. Before storing, an aliquot of the cecal content used in the inoculation strategy experiment (from a 40-week-old Lohmann white chicken) was used to develop the microbial culture inoculum. The aliquot was serial diluted in sterile 1 x phosphate-buffered saline (PBS) and plated on fastidious anaerobe agar (Neogen, US), blood heart infusion agar (Oxoid, CA), Wilkins-Chalgren anaerobe agar (Oxoid, CA), de Man, Rogosa and Sharpe agar (BD Difco™, US), and reinforced clostridial agar (BD, US), and incubated at 37°C. After 48 h, plates containing separated colonies were washed by adding sterile 1 x PBS. From each media plate, 2 ml of 1 x PBS with microbial cells were collected and pooled to generate the inoculum. The pooled microbial culture inoculum was mixed with LCY supplemented with 0.05% L-cysteine and 50% glycerol (final concentration, 25%), aliquoted in 5-ml tubes, and stored at -80°C until use. In all experiments, the frozen inocula were transported and kept on dry ice until the inoculation was performed. Individual aliquots were thawed at room temperature and vortexed before being inoculated to one isolator at a time to minimize exposure to air.

4.2.3. Experimental design

Inoculation strategies experiment. In the first trial (trial A), 90 one-day-old chicks with an average body weight (BW) of 48.29 ± 3.55 g (mean ± standard deviation (SD)) were randomly distributed to 30 isolators (3 birds per isolator), and assigned to one of the following four treatments: cecal contents (from a 40-week-old Lohmann white chicken) delivered via oral gavage (ceca_gavage, 7 isolators) or spraying into bedding (ceca_spray, 5 isolators); microbial culture derived from cecal contents delivered via oral gavage (culture_gavage, 7 isolators) or spraying into bedding (culture_spray, 5 isolators). The control group (control, 6 isolators) received sterile LCY supplemented with 30% glycerol and 0.05% L-cysteine via oral gavage. Birds receiving treatments via oral gavage got 150 µl of inoculum. For treatments using spray technique, 450 µl of cecal content or microbial culture were spread as coarse droplets on top of the bedding material using a syringe. The results indicated that the spray treatments using 450 µl of inocula failed to promote microbial colonization, therefore the experiment was repeated (trial B) using 75 one-day-old chicks with an average BW of 47.06 ± 3.69 g (mean ± SD) that were randomly distributed into 25 isolators (3 birds per isolator, 5 isolators per treatment) and assigned to the treatments described above except that the volume of inocula sprayed into bedding was increased to 2 ml. Birds were weighed weekly and the lightest bird from each isolator was euthanized at the end of each week to ensure adequate floor space for the remaining birds. At the end of the experiment, samples were collected and analyses were performed on the heaviest bird from each isolator.

Cohousing experiment. Six one-day-old chicks with an average BW of 39.95 ± 4.03 g (mean ± SD) were housed in three polycarbonate isolators with micro-filter tops. In each isolator, one bird was selected as the "seeder" bird and the other was called the "cage mate". The seeder bird received 150 µl of cecal contents obtained from a healthy backyard chicken (eA) by oral gavage, whereas

its cage mate remained untreated. Another three isolators contained two chicks that were inoculated with sterile LCY (150 µl) via oral gavage and assigned as the control group. Birds were weighed at 7 and 14 days old, and cecal samples from all birds were collected at 14 days old.

Competition experiment. Eighty-one one-day-old chicks with an average BW of 38.88 ± 4.06 g (mean ± SD) were randomly distributed into 27 isolators (3 birds per isolator, 3 isolators per treatment) and received cecal contents (150 µl) obtained from intensively (donor birds iA and iB) or extensively raised chickens (donor birds eA and eB), or a mixture containing cecal contents from intensively and extensively raised chickens via oral gavage (iAeA, iAeB, iBeA, iBeB). The control group received sterile 150 µl LCY supplemented with 30% glycerol and 0.05% L-cysteine. Birds were weighed once a week, and the lightest bird from each isolator was euthanized on day 7. The two remaining birds from each isolator were euthanized and cecal samples were collected on day 14.

4.2.4. Comparison of the microbiota of experimental birds and commercial broilers

The birds used in the present study were housed in microisolators to reduce the effects of environmental uncontrolled variables that could affect microbiota composition and confound the results, as this could hinder the ability to observe consistency across our experiments. To determine if the experimental housing conditions resulted in experimental birds harboring aberrant microbiota that would not adequately resemble the microbiota of broilers in commercial settings, we compared the microbiota of the birds used in the inoculation strategies experiment to the microbiota of intensively raised 21-day-old broilers from two commercial farms. Broilers in Farm 1 (n = 8) were Ross 308 provided with bacitracin in feed throughout the production cycle; and birds from Farm 2 (n = 5) were Ross 708 provided with salinomycin and bacitracin in feed

throughout the production cycle. These commercial broilers were euthanized on farm and cecal samples were collected in sterile tubes, transported in dry ice, and stored at -80°C until processing.

4.2.5. Sample collection

Samples were collected from one bird per isolator at 21 days of age in the inoculation strategies experiment. In the cohousing and competition experiments, samples were collected from two birds per isolator at 14 days of age. Blood was collected by cardiac puncture and plasma was harvested by centrifugation at 1,000 x g for 15 min at 4°C and stored at -80°C until further analysis. Cecal tissue and approximately 1 g of cecal digesta were collected from each bird, snap-frozen in liquid nitrogen and stored at -80°C. Segments of ileum (0.5 cm proximal and 0.5 cm distal to the Meckel's diverticulum) were collected into 10% neutral buffered formalin solution for histological analysis. All sampling procedures were conducted in a biosafety cabinet.

4.2.6. DNA extraction and 16S rRNA amplicon sequencing analysis

A QIAamp DNA stool mini kit (Qiagen Inc., US) was used to extract DNA from cecal digesta and microbial culture according to manufacturer's instructions with an additional bead-beating step. Briefly, approximately 100 mg of cecal contents or cell pellets collected after centrifugation of microbial culture at 20,000 x g for 5 min were mixed with 1 ml InhibitEx® buffer and 7 to 10 2.0 mm garnet beads (BioSpec Products, Bartlesville, OK) in a screwcap 2 ml tube. All samples were homogenized and lysed by bead-beating twice at 6.0 m/s for 30 s (FastPrep-24TM 5G, MP Biomedicals). Purity of extracted DNA was assessed using Nanodrop™ 2000 spectrophotometer (Thermo Scientific™) and concentrations were determined using Quant-iT™ PicoGreen dsDNA assay kit (Thermo Scientific™). Amplicon libraries were generated following

the Illumina 16S Metagenomic Sequencing Library Preparation protocol targeting the V3-V4 region of the 16S rRNA gene. Paired-end sequencing runs were performed on an Illumina MiSeq Platform (Illumina Inc, San Diego, CA) using 2 x 300 cycles.

Raw sequences were processed using Quantitative Insight into Microbial Ecology 2 (QIIME 2 v2021.4) (15). Pairing, denoising, de-replication and chimera filtering were performed using DADA2 (16). Forward and reverse sequences were truncated at 270 and 220 base pairs, respectively, based on median quality score. Sequences with more than 6 expected errors were discarded. Sequence alignments and the generation of phylogenetic trees were performed using mafft (17) and fastTree methods (18). The q-2-feature-classifier plugin (19) was used to assign taxonomy to amplicon sequence variants (ASVs) using a Naïve Bayes classifier (20) pretrained on SILVA 138 QIIME compatible database which was set to cluster sequences at 99% identity using majority taxonomy strings (21).

The phyloseq (22), microbiome (23) and qiime2R (24) packages in R (25) were used for downstream analysis. All ASVs assigned to Mitochondria family, Chloroplast order, Archaea kingdom or Unassigned were removed from the dataset. Rarefaction curves were generated using vegan package (26) and samples were normalized by rarefying reads at an even count for all the analysis. Alpha-diversity was evaluated using Chao1, Simpson and phylogenetic diversity indices. Beta-diversity was evaluated using Bray-Curtis distance matrix and principal coordinates analysis (PCoA). Hierarchical clustering was based on Bray-Curtis distance matrix and Ward method on squared dissimilarities (stats package). To test for differentially abundant features, ASVs exhibiting the same taxonomy string were combined using *tax_glom* function from phyloseq package (as a result, "taxa" can refer to differences at species, genus, family, and order levels), and analyses were performed at the taxonomy (taxa) level using limma-voom (27, 28), DESeq2

with apeglm and FDR correction (29, 30), and ANCOM-BC methods (31). To reduce the occurrence of type I error, features were considered as differentially abundant among treatments only if consistent differences were detected by all three methods. For DESeq2 analysis, a $\log_2$-fold change higher than 2.5 or lower than -2.5 with an adjusted *p*-value lower than 0.05 was considered as significant. Colonization efficiency was determined based on the number of overlapping ASVs between each inoculum and the gut microbiota from birds receiving the inoculum (32). The ASVs that were present in the control birds were considered as baseline microbiota and not included in the colonization efficiency calculations. Taxonomic core microbiota was defined as taxa present in more than two samples, with at least 1% abundance in any sample and with average relative abundance greater than 0.2%. In addition, taxa present in at least 80% of birds after the exclusion of taxa that had less than 10 reads in total were also considered as core taxa of the chicken microbiota. Figures showing results for alpha-diversity, beta-diversity, taxonomy plots and differential abundance analysis were generated using ggplot (33), ggpubr (34) and microbiome packages, visualization of overlapping ASVs between groups were created using ggvenn package (35). Heatmaps figures were generated using pheatmap package (36). Bar plots indicating the relative abundance of ASVs were generated using Microsoft Excel (37).

4.2.7. Histology

Formalin-fixed ileal tissues were embedded in paraffin and sectioned at 5 μm. Slides were stained with hematoxylin-eosin solution. Images were taken by Evos® FL Auto Imaging System (Thermo Scientific™). Representative cross-sections of each sample were selected based on the presence of intact and well-positioned lamina propria and villi, and measurements were taken from

one selected field that contained at least three well positioned and intact villi and crypts, at 40X magnification.

4.2.8. Cytokine and chemokine analyses

Cecal tissues were ground to fine powder using mortars and pestles chilled with liquid nitrogen. Ground tissue was mixed with radioimmunoprecipitation assay buffer (Thermo Scientific™) with 1% Halt™ protease inhibitor cocktail (Thermo Scientific™), incubated on ice for 25 min, and lysed by homogenized at 6.0 m/s for 10 s (FastPrep-24TM 5G, MP Biomedicals). The lysates were centrifuged at 10,000 x g for 15 min at 4°C. Protein concentration in the supernatant was quantified using Pierce™ BCA protein assay kit (Thermo Scientific™) following the manufacturer's protocol. Levels of interferon (IFN)-α, IFN-γ, interleukin (IL)-2, IL-6, IL-10, IL-16, IL-21, macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein(MIP)-1β, MIP-3α, CCL5/regulated on activation, normal T cell expressed and secreted (RANTES) and vascular endothelial growth factor A (VEGFA) were determined in tissue homogenates and plasma using ™Featured - Chicken cytokine/chemokine 12-Plex Assay (Eve Technologies Corporation, CA).

4.2.9. SCFA quantification

Cecal digesta was mixed with 25% phosphoric acid (w/v 1:4), homogenized by vortexing and centrifuged at 15,000 x *g* for 10 min at 4 °C. The supernatant was filtered through a 0.45 µm syringe filter and isocaproic acid (24.5 µmol/ml) was added at a 1:4 ratio to the filtered supernatant as an internal standard. Samples were analyzed in a Varian 430 Gas Chromatography (Varian inc.,

USA) with flame ionization detector using helium as the carrier gas and Stabilwax-DA 30 m x 0.53 mm ID, 0.5 μm df (Restek corp, Bellefonte, PA, USA) capillary columns.

4.2.10. Statistical analysis

At each time point, BW was analyzed using a linear mixed-effects model considering treatment as a fixed effect and initial BW as a random effect. Adjusted means were compared using one-way analysis of variance (ANOVA) followed by Tukey HSD test for pairwise comparisons. Alpha-diversity, histological measurements, cytokines/chemokines and SCFA data were analyzed using one-way ANOVA and Tukey HSD test when data were normally distributed; and using Kruskal-Wallis and Wilcoxon rank sum test with FDR adjustment if data were not normally distributed. Beta-diversity distances matrices were analyzed using permutational multivariate analysis of variance (adonis function/PERMANOVA) to test for differences in the distances to centroids and dispersion of the groups (vegan package).

4.3. RESULTS

4.3.1. Bacteroidetes dominated the cecal microbiota of inoculated birds

In trial A and B of inoculation strategies experiment, chicks were exposed to either cecal contents or microbial culture derived from cecal contents via oral gavage or spraying into bedding. The only difference in experimental procedures between the two trials was the volume of inoculum sprayed in bedding (450 μl in trial A vs. 2 ml in trial B). The 16S rRNA gene amplicon sequencing of cecal contents and the pooled microbial culture derived from cecal contents generated 45,111 and 36,497 reads, respectively, that were assigned to 745 ASVs and 264 taxa. From the total ASVs detected, 78% were found exclusively in the cecal content, 12% were found exclusively in the

microbial culture, and only 10% of the total number of ASVs were shared between cecal contents and the microbial culture. The percentage of taxa shared between the cecal contents and the microbial culture represented 23% of the total number of taxa. Comparisons of inoculum composition at the family level indicated that microbial culturing resulted in a reduction in the relative abundance of *Acidaminococcaceae*, *Bacteroidaceae*, *Prevotellaceae*, *Rikenellaceae*, and *Succinovibrionaceae*; and in an expansion of *Fusobacteriaceae*, *Lactobacillaceae*, and *Veillonellaceae* in comparison to cecal contents.

After inoculation in trial A, ceca_gavage birds showed lower BW at 7- ($p < 0.001$) and 14- ($p = 0.004$) days-old compared to birds from other treatments; however, there was no significant difference in BW at day 21 among treatments ($p = 0.370$). In trial B, there was no difference in BW at all timepoints, indicating a small impact of inoculation on chicken growth.

Cecal microbial communities at day 21 were characterized using 16S rRNA gene amplicon sequencing. In trial A, an average 23,361 ± 8,946 reads (n = 29) were generated ranging from 9,557 to 45,111 reads per sample, which were assigned to 1,491 ASVs and 374 taxa. The ceca_gavage treatment showed greater microbial richness (Chao1 index) than that in control and culture_spray treatments ($p < 0.001$), and higher PD than birds in control ($p = 0.025$) and ceca_spray ($p = 0.006$) treatments. In trial B, an average 20,792 ± 11,558 reads (n = 27) were generated ranging from 6,550 to 45,111 reads per sample, which were assigned to 1,485 ASVs and 372 taxa. Consistently, birds in the ceca_gavage treatment showed the greatest microbial richness (Chao1 index) and PD among treatments. In trial B, all treated groups presented higher PD than the control ($p < 0.013$) indicating that all treatments were effective in increasing taxonomic diversity within the cecal microbial community. Inocula treatments caused significant shifts in microbiota composition in both trials (trial A: $p = 0.001$, $R^2 = 0.43$; trial B: $p = 0.001$, $R^2 = 0.47$).

Pairwise comparisons indicated that all groups were significantly separated from each other in trial A (Figure 4.2A). In trial B, a similar clustering pattern was exhibited, except for no significant difference between ceca_spray and ceca_gavage groups ($p = 0.077$) (Figure 4.2B).

Hierarchical clustering analyses of trial A showed a separation of samples into two main clusters with one containing all control samples and most birds receiving spray treatments, and the other cluster containing inocula (cecal contents and microbial culture) and birds that received inocula via gavage technique. In trial B, control birds were separated from birds in other groups receiving inocula treatments. Within the cluster containing birds that received inocula, the mode of delivery caused a further separation between birds that received oral gavage and birds that received spray (Figure 4.3A). Bacteroidetes were highly abundant in birds inoculated via oral gavage and in birds inoculated via spray with an increased volume, reaching an average of 52% and 58% of microbial communities, respectively. Meanwhile, birds from the control treatment in both trials and most birds exposed to spray treatments in trial A exhibited a higher relative abundance of Firmicutes. The average relative abundance of Firmicutes in control birds was 80%, whereas in inoculated groups the average was less than 30% (Figure 4.3B). These results indicated that inocula of cecal contents and microbial cultures derived from cecal contents are both effective in increasing alpha diversity, changing the microbial community composition, and favoring the predominance of Bacteroidetes.

A total of 78 taxa were identified as core members of the microbiota. Several bacterial genera were consistently enriched in birds treated via oral gavage (ceca_gavage and culture_gavage) compared to control birds. Namely, gavage-treated birds had enriched *Alistipes*, *Anaerobiospirillum*, *Bacteroides plebeius*, *Barnesiella*, *Mailhella*, *Mediterranea massiliensis, Megamonas*, *Olsenella, Oribacterium*, *Parabacteroides*, and *Phascolarctobacterium*; whereas

control birds had enriched genera *Escherichia-Shigella* (Figure 4.4.A, 4.4.B). In trial A, the ceca_spray and culture_spray treatments had minor effects on taxa enrichment. In trial B, birds receiving spray treatments (ceca_spray and culture_spray) had enriched *Bacteroides sp. SB5*, *Mediterranea massiliensis*, *Megamonas*, *Olsenella*, and *Phascolarctobacterium*, whereas birds in the control group had enriched *Escherichia-Shigella* and *Subdoligranulum* (Figure 4.5.A and Figure 4.5.B). Comparisons between ceca_gavage and culture_gavage treatments indicated that culture_gavage consistently promoted an enrichment of *Sutterella*, whereas ceca_gavage promoted an enrichment of members from the family *Ruminococcaceae*, the phylum Patescibacteria, the order Clostridiales, genera *Helicobacter* and *Sphaerochaeta* in both trials (Figure 4.6). Comparisons between the gavage and spray treatments applied on trial B show that few taxa were differentially abundant in birds receiving ceca_gavage and culture_gavage compared to birds receiving ceca_spray and culture_spray, respectively (Figures 4.7.A and 4.7.B), indicating that both gavage and spray can be used as effective delivery routes provided that a sufficient volume of spray is applied.

Colonization efficiency results indicated that 23.1% and 25.3% of the ASVs present in ceca contents were also detected in ceca_gavage birds in trials A and B, respectively. For ceca_spray birds, the percentage of ASVs shared with the inoculum of ceca contents was 5.5% in trial A, and reached 13.3% in trial B when an increased volume of inoculum was sprayed. In trials A and B, the percentage of ASVs present in the inoculum of microbial cultures that were also detected in culture_gavage birds was 30.5% and 37.2%, respectively. The percentage of taxa presented in the inoculum of microbial cultures that were also detected in culture_spray birds was low in trial A (14.0%), but was improved in trial B (33.5%). We also evaluated the relative abundance of ASVs that are either unique or shared between inoculum treated birds and baseline microbiota.

Approximately 58% of the microbiota present in the cecal content inoculum was also detected in inoculated birds, and therefore constitute the portion of the microbial community that was successfully transferred to the birds. In addition, 13% of the microbiota in the cecal content inoculum was detected in the baseline gut microbiota and in inoculated birds, representing the percentage of the microbial community that could be originating both from the baseline microbiota and the inocula. Approximately 30% of the microbiota of the cecal content inoculum was not detected in inoculated birds, which indicate that more than half (and potentially 71%) of the microbial community in the cecal content inoculum was able to colonize the inoculated birds. Within the microbiota of inoculated birds, 60% of the cecal microbiota community was composed of ASVs that originated from the inocula, suggesting that baseline microbiota was replaced by bacteria that are likely more adapted to the chicken gastrointestinal tract.

4.3.2. The microbiota of control birds in the inoculation strategies experiments have lower Bacteroidetes and cluster closely to the microbiota of broilers in commercial farms

We compared the microbiota of experimental chicks that were exposed to ceca_gavage and culture_gavage treatments (exposed) to the microbiota of control chicks (control) and broilers of same age reared in two intensive commercial farms (Farm 1 and Farm 2). Clustering analysis shows a separation of the birds into two main branches, one containing all the experimental chicks exposed to the gavage treatments, and the other group containing the experimental controls and broilers in commercial farms. Firmicutes dominated the microbiota of control and commercial birds, whereas the microbiota of exposed birds is dominated by Bacteroidetes. The results indicated that, although reared in conditions that would be deemed extremely hygienic, the control birds in the present study harbor a microbiota similar to that of birds in commercial settings. One

major difference is the lower level of Bacteroidetes observed in control groups (absent in half of the birds and presenting relative abundances ranging from 0.04% to 0.69%) compared to commercial farms (absent in two birds and presenting relative abundances ranging from 0.23% to 43.15%). Meanwhile, all exposed birds were shown to harbor Bacteroidetes with relative abundances ranging from 31.51% to 71.85%. Differential abundance analysis indicated 29 taxa to be different between commercial broilers and our exposed birds; whereas 11 taxa were different between commercial and control birds. Interestingly, the microbiota of exposed birds showed enrichment of *Bacteroides plebeius*, *Barnesiella*, *Desulfovibrio*, *Megamonas*, *Megasphaera*, *Mediterranea massiliensis*, *Olsenella*, *Oribacterium*, *Parabacteroides* and *Phascolarctobacterium* compared to the microbiota of commercial birds, which showed enrichment of several members of the Firmicutes, alongside enrichment of *Escherichia-Shigella*. Enrichment of the same taxa was observed when comparing the microbiota of exposed and control birds; indicating consistency between the microbiota of control and commercial intensively raised broilers.

Histomorphology was evaluated in ileal tissues from 21-day-old broilers in trial B. Ceca_gavage birds had higher villus height and crypt depth than that in culture_spray ($p = 0.014$ and $p = 0.001$, respectively) and the control ($p = 0.021$ and $p = 0.004$, respectively) treatments. Villus width was higher in ceca_gavage, ceca_spray, and culture_gavage treatments compared to the control group ($p = 0.047$, $p = 0.005$, and $p= 0.020$, respectively). Ceca_spray birds showed deeper crypts than control ($p < 0.001$) and culture_spray ($p < 0.001$) treatments. The villus height to crypt depth ratio was lower for ceca_spray birds compared to the control ($p < 0.001$) and culture_spray ($p < 0.001$) treatments (Table 2.1).

Levels of cytokines/chemokines in cecal tissue homogenates and plasma samples from 21-day-old birds from trial B were evaluated. Cecal samples from birds in ceca_gavage,

culture_gavage, and culture_spray groups had higher levels of IL-6 ($p < 0.001$) than that of birds in the control group. Birds in ceca_gavage and culture_spray treatments had higher IL-10 ($p = 0.002$) than that in the control group. Levels of VEGF were higher in the ceca of birds in ceca_spray compared to culture_spray group ($p = 0.044$) (Table 2.2). There were no differences in the cecal concentration of IFN-α, IFN-γ, IL-16, MIP-1β, MIP-3α, M-CSF, and RANTES, nor in the concentration of IFNγ, IL-16, MIP-1β, MIP-3α, and M-CSF in plasma (Table S4). The levels of IFN-α, IL-2, IL-6, IL-10, IL-21, RANTES, and VEGF were below the detection range in plasma samples, and the levels of IL-2 and IL-21 were below the detection range in tissue homogenates.

Levels of propionate in cecal contents were higher in inoculated birds compared to the control group ($p = 0.027$). Valerate was higher in ceca_gavage, ceca_spray, and culture_gavage birds compared to the control group ($p = 0.024$). No significant differences between acetate, isobutyrate, butyrate, and isovalerate levels were observed (Table 4.3).

4.3.3. Seeder birds effectively transferred their microbiota to cage mates

This experiment was performed to test if the microbiota of one chick (the seeder) could be effectively transmitted to another chick (the cage mate) housed in the same isolator. We inoculated one-day-old seeders with cecal contents obtained from a healthy backyard 40-week-old chicken donor (eA) by oral gavage and used 16S rRNA gene amplicon sequencing to evaluate the cecal microbiota of each seeder and its cage mate after 2 weeks. An average of 38,621 ± 29,780 reads (n=12) were generated and assigned to 1,116 ASVs and 332 taxa. There were no significant differences in BW at days 7 and 14 after inoculation treatments. Chao1 and Shannon indices were lower in the control birds compared to seeders and cage mates ($p < 0.001$ and $p = 0.005$, respectively). The inoculation caused significant shifts in cecal microbial community structures

between birds that were exposed to the inoculum and the control birds (p = 0.006, R^2 = 0.45). Specifically, control birds clustered separately from seeder birds and their cage mates (p = 0.017). This was further confirmed by hierarchical clustering analysis, which showed a separation of samples into two main clusters, with one containing all control samples and another containing the seeder birds, their cage mates and the inoculum eA (Figure 4.8A). Similar to the observed results in the inoculation strategies experiment, members of the phylum Bacteroidetes dominated the cecal microbial community of birds that were exposed to the inoculum, whereas Firmicutes and Proteobacteria were dominant in the microbiota of control birds (Figure 4.8B).

A total of 61 taxa were detected as core in this cohousing experiment, making up 91.6% to 99.2% of the cecal microbiota in birds. In comparison to control birds, seeder birds and cage mates had enriched *Alistipes*, *Barnesiella*, *Bifidobacterium*, *Collinsella massiliensis* and *Subdoligranulum* (Figure 4.9). Seeder birds presented enriched *Lactobacillus murinu*s and cage mates presented enriched *Lactobacillus salivarius* compared to control. No taxa were found to be differentially abundant between seeders and cage mates, indicating that the cecal microbiota of birds in the same cage remained highly similar to each other. Around 64% of the microbial community in the inoculum was composed of ASVs that were unique to the inoculum, thus failed to be transferred to the inoculated birds. On the other hand, an average of 52% of the inoculated birds' microbiota was composed by ASVs originating from the inoculum, indicating that, once transferred, the ASVs from the inoculum were able to colonize and multiply, regardless of the relatively small percentage of ASVs being transferred. We concluded that, in this cohousing experiment, at least 29% of microbes in the inoculum were successfully transferred to birds, and once transferred they made up more than half of the microbial population in the birds. Additionally,

the microbiota of the cage mates mimicked that of the seeders, indicating that cohousing is an effective way to promote microbiota transplantation.

4.3.4. Microbes from chickens raised extensively effectively colonized the modern broilers' gut

The competitive colonization study was conducted to evaluate the colonization ability of bacteria unique to extensive or intensive systems. Day-old chicks received cecal contents obtained from intensively or extensively raised chickens, or a mixture containing cecal contents from both origins via oral gavage. Cecal contents of two chickens from extensive systems (eA and eB) and two chickens from intensive systems were used (iA and iB). Inocula were applied individually (eA, eB, iA, and iB) or as a mixture (eAiA, eBiA, eBiA, and eBiB). An average 43,717 ± 27,743 reads (n = 57) were generated and assigned to 4,144 ASVs and 555 taxa. At day 7 and 14 after the inoculation, birds inoculated with eB had lower BW than birds from the control ($p = 0.009$), iA ($p = 0.023$) and eAiA treatments ($p = 0.023$). The cecal microbial communities were analyzed using 16S rRNA gene amplicon sequencing. All alpha-diversity indices measured were higher in birds that received inocula from extensively raised chickens (eA and eB) compared to the control birds. Chao1 and Simpson indices were significantly higher in birds that received mixtures (iAeA, iAeB, iBeA, and iBeB) compared to the control group, indicating that the inoculation with cecal contents from extensively raised chickens tended to consistently increase the biodiversity of the cecal microbiota. Inoculation treatments caused significant shifts in microbiota composition ($p = 0.001$, $R^2 = 0.57$). Hierarchical clustering analysis showed that all control birds clustered in a single clade that was separated from samples of inoculated birds. Within the clade containing inoculated birds there were four main clades: one clade included the inoculum iA and birds inoculated with iA and iAeA. One consisted of the inoculum iB and birds inoculated with iB and iBeA. A third clade

contained the inoculum eA and the birds inoculated with eA. The last clade included the inoculum eB, birds inoculated with eB, and birds inoculated with mixtures of iAeB and iBeB (Figure 4.10.A). These results suggested that microbes from the eA cecal contents, which originated from a 40-week-old bantam backyard chicken, were able to colonize when inoculated alone, but failed to thrive when the inoculum was mixed with cecal contents obtained from intensively raised broilers (iA and iB). On the other hand, the eB microbiota, harvest from the 35-day-old Cornish cross from an organic system, was more able to colonize the chicken gut compared to iA and iB microbes. The microbiota of control birds was dominated by Firmicutes and Proteobacteria, whereas inoculated birds showed a predominance of Bacteroidetes (Figure 4.10.B), which was consistent to the observed results in the inoculation strategies and cohousing experiments.

A total of 96 taxa were identified as core members in the competition experiment, making up 60% to 98% of the microbial community in all birds. Consistent with previous findings, the microbiota of the inoculated groups was dominated by Bacteroidetes and showed lower relative abundance of *Escherichia-Shigella* than the microbiota of control birds (Figure S17). Birds exposed to eA and eB showed higher relative abundance of *Enorma*, *Phascolarctobacterium,* and *Mediterranea massiliensis*, as well as members of the *Flavobacteriaceae* family. Birds exposed to iA and iB showed higher relative abundance of *Bacteroides dorei*, *B. fragilis*, *Barnesiella*, *Butyricimonas*, *Campylobacter jejuni*, *Coprobacter*, *Parabacteroides johnsonii*, *Escherichia-Shigella*, and members of the *Ruminococcaceae* family (Figure 4.11). In birds that received eAiA and eBiA, the percentage of ASVs that were successfully transferred to birds was higher for the iA inoculum compared to the eA and eB inocula. In birds treated with eAiA and eBiA, 30.01% and 32.40% of ASVs detected in the iA inoculum were also detected in the chicken gut, respectively; while only 16.25% and 24.94% of the ASVs detected in eA and eB, respectively,

were transferred to birds. In birds inoculated with eAiB and eBiB, the percentage of ASVs from the iB inoculum detected in birds was 28.49% and 33.43%, respectively; while for eA and eB inocula the percentages of successful transferring were 13.06% and 23.16%, respectively (Figure S18). Although the number of ASVs transferred from extensively raised chickens was lower than that transferred from intensively raised chickens, the average relative abundance of ASVs originated from extensively raised chickens was higher in the microbiota of birds inoculated with eAiA, eBiA, and eBiB (Figure 4.12).

4.4. DISCUSSION

Previous studies indicated that intensive poultry production practices may hinder the development of the broiler gut microbiota and impair resistance to pathogens (13). For more than 60 years, it has been consistently demonstrated that exposure of newly hatched chicks to intestinal contents from mature birds or to competitive exclusion products containing mixtures of bacteria harvested from poultry ceca can reduce pathogen establishment (38-42) and potentially improve poultry performance (43). The earlier studies had limited ability to characterize the microbiota composition of inoculated birds, which can now be done with the advancement of sequencing technology. To date, several studies have characterized the microbial composition of microbial inocula and the microbiota of inoculated birds (44-48). However, observed results vary due to differences in experimental design and due to host and environmental factors that affect microbiota composition (49-54). Variation in the microbiota composition is also reported in birds from the same hatchery reared in the exact same conditions and analyzed using the same methods (55). The lack of contact between chicks and hens coupled with the stochastic nature of microbial

colonization is likely to amplify the natural variation in the microbiota of newly hatched chicks and negatively affect the reproducibility of results between different batches of birds (55, 56).

The experimental chicks used in the present study were housed in microisolators to lower the chances of cross-contamination and to minimize variation in microbiota composition due to environmental factors. We inoculated three batches of birds with inocula from various sources and observed that the microbiota of inoculated birds presented a similar profile, despite the distinct composition of inocula introduced (Figure 4.1B). We compared the microbiota of our experimental birds with the microbiota of intensively raised broilers of matching age from two commercial farms to determine whether this experimental system is reasonably representative of birds in a conventional environment. The microbiota of experimental control birds was similar to that of birds in commercial farms, with a predominance of Firmicutes; while the microbiota of birds that were exposed to inocula was dominated by Bacteroidetes. We observed consistency between taxa that were enriched in experimentally exposed birds compared to birds in the control groups and also to broilers from commercial farms, indicating that the use of microisolators is a reasonable system to study the microbiota of poultry. Despite the usefulness of this controlled model, future validation in a less controlled setting is warranted.

The results from the inoculation strategies experiments showed that the cecal content from a 40-week layer and the microbial culture obtained from that had distinct microbial composition, likely due to differences on the ability of bacterial species to grow on media, which led to the reduction in the relative abundance of those with fastidious growth requirements and the increase of the less demanding species. Despite the small number of ASVs shared by both inocula, the birds inoculated with either cecal contents or microbial cultures presented similar microbiota, indicating that microbial cultures mimicked most of the changes induced by cecal contents. These findings

highlight one of the limitations of 16S rRNA sequencing data, which provides relative, not absolute, abundance of features in a sample and is limited by the instrument sequencing capacity (57). Consequently, species that are present at low abundance might be undetected in favor of the highly abundant ones. When the microbial culture was introduced back to the cecal environment, species with low abundance in the culture were able to multiply and therefore became detectable. On the other hand, species that easily grow in culture do not necessarily grow well in the gut and their lower presence could result in them not being detected. Birds exposed to microbial culture or cecal contents via spray or gavage consistently showed increased *Mediterranea massiliensis*, *Megamonas*, *Olsenella* and *Phascolarctobacterium* compared to control birds. The bacterial taxa consistently shown to colonize the chicken gut after a single exposure are likely highly adapted to the avian intestinal environment and potentially could be used to develop probiotic products, thus these taxa were flagged as of interest for future studies.

The microbiota of chicks inoculated via gavage with microbial culture or cecal contents presented enrichment of *Alistipes*, *Anaerobiospirillum*, *Bacteroides plebeius*, *Barnesiella*, *Mailhalla*, *Mediterranea massiliensis*, *Megamonas*, *Olsenella*, *Oribacterium*, *Parabacteroidetes* and *Phascolarctobacterium*. The genera *Alistipes*, *Bacteroides*, and *Barnesiella* were previously shown to comprise 75% of the microbial community derived from serial passages of cecal contents through multiple generations of chickens (58), indicating that they are stable colonizers of the chicken gut. Interestingly, birds exposed to used litter from a commercial flock had increased abundance of *Alistipes* and *Barnesiella* despite these bacteria not being detected in the litter (58), indicating their ability to colonize even when their presence in the inoculum is below detection levels. *Parabacteroides* was identified as core genera of the chicken microbiota (59) that can be efficiently transferred from parents to offspring (60), is enriched in birds exposed to microbial

inocula (58) and negatively associated with *Campylobacter* (11). Additionally, *Alistipes*, *Bacteroides*, *Barnesiella*, and *Mediterranea* isolates were shown to persist for at least one week after a single inoculation to day-old chicks (42), indicating their adaptability to the chicken gut. *Megamona*s, *Olsenella* and *Phascolarctobacterium* were shown to be effectively transmitted to chicks cohoused with an adult hen (60). *Megamonas* has been shown to inhibit *Salmonella* growth *in vitro* (61), and *Megamonas* isolated from wild poultry have been associated with a reduction in *Campylobacter jejuni* (62).

Besides enrichment of specific taxa, all inoculations performed in the three experiments were shown to promote an expansion of Bacteroidetes. Previous studies have also shown an increased relative abundance of Bacteroidetes as birds age, which is usually coupled with a decrease in Proteobacteria abundance; therefore, the predominance of Bacteroidetes could indicate maturation of microbiota communities and their ability to resist colonization by potential pathogens (45, 47, 60). The microbiota of commercial broilers seems to miss many Bacteroidetes species as observed in the control birds in our experiments and in other studies (2, 3). Our results indicated that Bacteroidetes were able to colonize when introduced a single time, on the first day of life, via oral gavage, spraying, or cohousing, despite being non-spore forming strict anaerobes with limited ability to survive in the environment. Chicks cohoused with a hen for 24 h present a higher abundance of Bacteroidetes at 7 days of age than control chicks that were not exposed to mature microbiota (60). This could indicate that age-related differences observed in the microbiota of commercial broilers are a consequence of delayed or a lack of contact with mature microbiota, and that Bacteroidetes might be particularly affected. Chicks harboring high levels of *Bacteroides* in the cecal microbiota have shown enriched polysaccharide degradation and SCFA production pathways, a decreased expression of pro-inflammatory IL-1β and an increased expression of IL-

10 compared to chicks with low *Bacteroides* levels (63). These findings warrant further investigation on the effects of introducing Bacteroidetes species to broilers in early life.

The observed changes in the microbiota composition were coupled with changes in host physiological and immune responses. Significant reductions in BW were observed in trial A of the inoculation strategies experiment in which ceca_gavage treated birds were on an average of 7% and 11% lighter than control birds on days 7 and 14, respectively. The BW was also lower in birds inoculated with the eB microbiota obtained from a broiler raised in a free-range organic system in the competition experiment compared to control birds. The impact of inoculations on BW may be attributed to mucosal and systemic immune activation induced by bacterial presence as a metabolic cost for the host (64, 65); to the utilization of dietary substrates by bacteria resulting in reduced nutrient availability (65); as well as to the reduced Firmicutes to Bacteroidetes ratio found in inoculated groups, which has been linked to lower ability of the host to acquire and store energy from the diet (66, 67, 68). In addition, chickens in the control group tended to present lower villus height, crypt depth and villus width compared to inoculated birds, especially to those that received cecal contents. These changes were compatible with decreased inflammation and cell turnover rate in control birds, and therefore led to the incurrence of a lower metabolic cost for microbial maintenance compared to inoculated chickens.

The increase in IL-6 and IL-10 observed in inoculated chicks was compatible with an activation of pro-inflammatory pathways and T regulatory cell responses to restore homeostasis, as seen in humans (69). Avian IL-6 mediates acute phase proteins production and fever (70, 71, 72), promotes antibody production (73), induces corticosterone release (74), and is elevated after infectious and inflammatory challenges (75-78). Meanwhile, IL-10 regulates the synthesis of pro-inflammatory cytokines (79), shifts the immune response towards a Th2 type response (80), and

has been linked to infection persistence (81) and disease burden (82). Heterophils of chickens resistant to extra-intestinal *Salmonella* infection have been shown to express higher IL-6 than the ones of susceptible chickens (82). On the other hand, higher IL-10 expression in blood and intestinal tissue has been associated with systemic infection, infection persistence, and poor prognosis after *Salmonella* and *Eimeria* infection (82-85). In humans, concomitant increases in plasma IL-6 and IL-10 have been linked to poor health outcomes (69, 86); however, studies on the dynamics of these two cytokines in chickens are scarce. Studies in mice indicated that commensal microbes are required for proper toll-like receptor activation and the production of intestinal IL-6, which is protective against intestinal injury (87, 88). In addition, monocolonization of germ-free mice with selected *Bacteroidales*, including *Alistipes* and *Bacteroides*, was shown to promote expansion of intraepithelial lymphocytes and IL-6 production (88). In chickens, changes in IL-6 and IL-10 levels have been shown after microbiota transplantation (89, 90, 9184) and infectious challenges (75, 92, 93), although the results may vary depending on experimental design and genetics of chickens (94).

Increase in propionate has been positively correlated with protection against infection challenges (95, 96, 97). In the present study, propionate producers (98-101), including Bacteroidetes, *Megamonas*, *Phascolarctobacterium*, and *Megasphaera,* were significantly increased in birds treated with microbial inocula compared to control birds, which might explain the observed increase in propionate levels. Valeric acid added to broiler diets has been shown to increase villus height to crypt depth ratio, reduce the incidence of lesions after a necrotic enteritis challenge and improve feed efficiency and BW (102). In the current study, valeric acid producers such as *Oscillibacter* and *Megasphaera* (103, 104) were enriched by inoculations. We speculated

that the increased concentration of these SCFAs would be beneficial in birds facing infectious challenges.

Across the three experiments, the following bacteria were consistently assigned as core members of the chicken gut microbiota: *Bacteroides gallinaceum*, *Bacteroides plebeius*, *Bacteroides salanitronis DSM18170*, *Mediterranea massiliensi*s, *Barnesiella*, *Alistipes*, *Parabacteroides*, *Mucispirillum*, *Enterococcus*, *Lactobacillus*, *Clostridiales vadinBB60* group, *[Ruminococcus] torques*, *Eisenbergiella*, *Sellimonas*, *Shuttleworthia*, *Butyricicoccus*, *Faecalibacterium*, *Flavonifractor*, Negativibacillus, *Ruminiclostridium 9*, *Ruminococcacea UCG-014*, *Subdoligranulum*, *Phascolarctobacterium*, *Gorbachella massiliensis*, *Erysipelatoclostridium*, *Dialister*, *Megamonas*, *Escherichia-Shigella* and *Synergistes*. Identification of core taxa can assist researchers to define a healthy microbiota in chickens, identify chicken-adapted bacteria, and guide the development of gnotobiotic chicken models to explore causal links between the gut microbiota and host responses (105, 106, 107). *Bacteroides plebeius, B. gallinaceum* and *B. salanitronis* were previously shown to be poultry adapted species (108). *B. plebeius* was shown to be increased in the microbiota of gavage-treated birds in the inoculation strategies experiment compared to control birds and to broilers from commercial farms. Broilers in commercial farms showed enrichment of *Bacteroides dorei* compared to gavage-treated birds; and *B. dorei* was also shown to be enriched in the microbiota of birds inoculated with cecal contents obtained from broilers in intensive rearing systems. This is of interest because *B. dorei* was shown to be more abundant in the microbiota of humans than in the microbiota of hens (108); and the higher abundance of *B. dorei* observed in the microbiota of intensively raised broilers could be related to the absence of chicken-adapted species within the environment. The fact that *B. plebeius* is enriched in our gavage-treated birds compared to broilers in commercial farms

suggests that *B. plebeius* can replace non-adapted species, such as *B. dorei*, if opportunity for contact is provided.

In the competition experiment we compared the colonization ability of complex communities of bacteria harvested from extensively and intensively raised chickens. Although there have been efforts to characterize the colonization ability of cecal communities or defined mixtures containing selected bacterial isolates (42, 45, 47, 48, 109), the inoculation of mixed communities containing microbiota from extensively and intensively raised chickens was not previously investigated. Understanding the difference in microbial colonization of inocula obtained from different systems is relevant since colonization with a host-adapted, naturally selected microbiota, may modulate immune responses that improve host disease resistance and survival (110), as demonstrated in mouse studies (111, 112). Additionally, microbiota from extensively raised chickens can present lower abundance of antimicrobial resistance genes (3) and higher antagonist activity against pathogens (113). Although the number of ASVs transferred from intensively raised donors to chicks was higher than that transferred from extensively raised donors, the microbiota of chicks colonized with mixed communities presented a higher relative abundance of ASVs originating from extensively raised donors. This is indicative that, despite some microbial taxa from extensively raised birds fail to colonize the gut, the ones that were transferred could efficiently multiply and establish within the gut. It is possible that extensively raised birds harbored transient bacteria originating from feed material and soil, which would explain the lesser number of ASVs from extensive systems detected in the gut of inoculated chicks. We emphasize that this was a small-scale study that tested only two inocula from intensive systems and two inocula from extensive systems. We observed that the inocula eA, which was harvested from a 40-week-old bantam backyard chicken was able to colonize when introduced alone, but was less efficient to

colonize than the cecal contents from intensively raised broilers when introduced as a mix. This could be due to the differences in age and genetics of the donor vs the inoculated chicks. Nonetheless, when considering only the inocula obtained from 35-day-old broilers (eB, iA, iB), the inoculum originating from the organic broiler (eB) colonized more efficiently than the two inocula obtained from intensively raised broilers. The results of this preliminary trial suggest a higher colonization ability of bacteria originating from extensive systems; but further studies of colonization ability of commensals sourced from chickens raised in different systems and their effects on bird physiology are necessary to determine what microbes are adequate for birds in commercial settings.

4.5. CONCLUSION

In the present study we demonstrated that day-old commercial broiler chicks exposed to cecal contents or microbial cultures were readily colonized by Bacteroidetes and present significantly higher abundance of *Alistipes*, *Bacteroides*, *Barnesiella*, *Mediterranea*, *Megamonas*, *Parabacteroides*, *Phascolarctobacterium*, and *Subdoligranulum* than control birds. These results were consistently observed using inocula harvested from different donors and introduced via gavage, spray, or cohousing methods, indicating that this set of bacteria are highly adapted and easily transmitted to the chicken host. Inoculated birds also present lower relative abundance of *Enterobacteriaceae*, namely *Escherichia-Shigella*, which can negatively impact chicken health and cause food-borne illnesses. These results indicated that microbiota transfer can potentially promote pathogen exclusion and affect bird physiology, and our findings will guide future research exploring the effects of selected bacterial isolates on the gut microbiota and physiology of birds subjected to infectious challenges, aiming to develop host-adapted probiotic products.

4.6. REFERENCES

1. Cressman MD, Yu Z, Nelson MC, Moeller SJ, Lilburn MS, Zerby HN. 2010. Interrelations between the microbiotas in the litter and in the intestines of commercial broiler chickens. Appl Environ Microbiol. 76(19):6572-82. doi: 10.1128/AEM.00180-10

2. Ferrario, C., Alessandri, G., Mancabelli, L., Gering, E., Mangifesta, M., Milani, C., Lugli, G.A., Viappiani, A., Duranti, S., Turroni, F., Ossiprandi, M.C., Hiyashi, R., Mackie, R., van Sinderen, D. and Ventura, M. 2017. Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses. Environ Microbiol, 19: 4771-4783. https://doi-org/10.1111/1462-2920.13943

3. Seidlerova Z, Kubasova T, Faldynova M, Crhanova M, Karasova D, Babak V, Rychlik I. 2020. Environmental Impact on Differential Composition of Gut Microbiota in Indoor Chickens in Commercial Production and Outdoor, Backyard Chickens. Microorganisms. 8(5):767. doi: 10.3390/microorganisms8050767

4. McBride, G., I. P. Parer, and F. Foenander. 1969. The social organization and behaviour of the feral domestic fowl. Anim. Behav. Monogr. 2:125–181. doi:10.1016/s0066-1856(69)80003-8.

5. Foster, K. R., J. Schluter, K. Z. Coyte, and S. Rakoff-Nahoum. 2017. The evolution of the host microbiome as an ecosystem on a leash. Nature. 548:43–51. doi:10.1038/nature23292.

6. Yeoman, C. J., N. Chia, P. Jeraldo, M. Sipos, N. D. Goldenfeld, and B. A. White. 2012. The microbiome of the chicken gastrointestinal tract. Anim. Health Res. Rev. 13:89–99. doi:10.1017/S1466252312000138.

7. Pan, D., and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 5:108–119. doi:10.4161/gmic.26945.

8. Hou Q, Kwok LY, Zheng Y, Wang L, Guo Z, Zhang J, Huang W, Wang Y, Leng L, Li H, Zhang H. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. Sci Rep. 2016 Nov 23;6:37376. doi: 10.1038/srep37376.

9. Wei S, Morrison M, Yu Z. 2013. Bacterial census of poultry intestinal microbiome. Poult Sci Sympos. 92:671–83. doi: 10.3382/ps.2012-02822

10. Chica Cardenas LA, Clavijo V, Vives M, Reyes A. Bacterial meta-analysis of chicken cecal microbiota. PeerJ. 2021 Jan 5;9:e10571. doi: 10.7717/peerj.10571. PMID: 33505795; PMCID: PMC7792525.

11. Ocejo, M., Oporto, B. & Hurtado, A. 2019. 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. Sci Rep 9, 2506 https://doi.org/10.1038/s41598-019-39323-x

12. Ballou, A. L., R. A. Ali, M. A. Mendoza, J. C. Ellis, H. M. Hassan, W. J. Croom, and M. D. Koci. 2016. Development of the chick microbiome: How early exposure influences future microbial diversity. Front. Vet. Sci. 3:1–12. doi:10.3389/fvets.2016.00002.

13. Nurmi, E., and Rantala, M. 1973. New aspects of *Salmonella* infection in Broiler production. Nature 241:210-211. doi: https://doi.org/10.1038/241210a0

14. Baldwin, S., R. J. Hughes, T. T. H. Van, R. J. Moore, and D. Stanley. 2018. At-hatch administration of probiotic to chickens can introduce beneficial changes in gut microbiota. PLoS One. 13:1–14. doi:10.1371/journal.pone.0194825.

15. Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F.

Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. Bin Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37:852–857. doi:10.1038/s41587-019-0209-9.

16. Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods. 13:581–583. doi:10.1038/nmeth.3869.

17. Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30:772–780. doi:10.1093/molbev/mst010.

18. Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS One. 5. doi:10.1371/journal.pone.0009490.

19. Bokulich, N. A., B. D. Kaehler, J. R. Rideout, M. Dillon, E. Bolyen, R. Knight, G. A. Huttley, and J. Gregory Caporaso. 2018. Optimizing taxonomic classification of marker-gene

amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 6:1–17. doi:10.1186/s40168-018-0470-z.

20. Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, E. 2011. Scikit-learn: machine Learning in Python. Journal of Machine Learning Research. 12:2825-2830.

21. Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41:590–596. doi:10.1093/nar/gks1219.

22. McMurdie, P. J., and S. Holmes. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 8. doi:10.1371/journal.pone.0061217.

23. Lahti, L., and S. Shetty. 2018. Introduction to the microbiome R package. Bioconductor. 1–71.

24. Bisanz, J., 2018. qiime2R: Importing QIIME2 artifacts and associated data into R sessions. Available at: https://github.com/jbisanz/qiime2R

25. RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

26. Oksanen J., Simpson G., Blanchet F., Kindt R., Legendre P., Minchin P., O'Hara R., Solymos P., Stevens M., Szoecs E., Wagner H., Barbour M., Bedward M., Bolker B., Borcard D., Carvalho G., Chirico M., De Caceres M., Durand S., Evangelista H., FitzJohn R., Friendly M., Furneaux B, Hannigan G., Hill M., Lahti L., McGlinn D., Ouellette M., Ribeiro Cunha E., Smith

T., Stier A., Ter Braak C., Weedon J. 2020. vegan: Community Ecology Package. R package version 2.5-7., https://CRAN.R-project.org/package=vegan.

27. Law, C. W., Y. Chen, W. Shi, and G. K. Smyth. 2014. Voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 15:1–17. doi:10.1186/gb-2014-15-2-r29.

28. Ritchie, M. E., B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, and G. K. Smyth. 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43:e47. doi:10.1093/nar/gkv007.

29. Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:1–21. doi:10.1186/s13059-014-0550-8.

30. Zhu, A., Ibrahim, J. G., Love, M. I. 2019. Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences, Bioinformatics, 35(12):2084–2092, https://doi.org/10.1093/bioinformatics/bty895

31. Lin, H., and S. Das Peddada. 2020. Analysis of compositions of microbiomes with bias correction. Nat. Commun. 11:1–11. doi:10.1038/s41467-020-17041-7.

32. Dong, S., B. H. Zeng, L. Hu, Y. Zhang, J. Xiong, J. Deng, L. Huang, Z. L. Liao, J. Wang, H. Wei, and X. Fang. 2021. Effect of a humanized diet profile on colonization efficiency and gut microbial diversity in human flora-associated mice. Front. Nutr. 8:1–14. doi:10.3389/fnut.2021.633738.

33. Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, https://ggplot2.tidyverse.org.

34. Kassambara A. 2022. ggpubr: 'ggplot2' Based Publication Ready Plots_. R package version 0.5.0, https://CRAN.R-project.org/package=ggpubr

35. Yan L. 2022. ggvenn: Draw Venn Diagram by 'ggplot2'. R package version 0.1.9.

36. Kolde, R., 2019. pheatmap: Pretty Heatmaps. R package version 1.0.12, https://CRAN.R-project.org/package=pheatmap

37. Microsoft Corporation. 2018. Microsoft Excel

38. Olesiuk, O. M., G. H. Snoeyenbos, and C. F. Smyser. 1971. Inhibitory effect of used litter on *Salmonella typhimurium* transmission in the chicken. Avian Dis. 15:118–124. doi:10.2307/1588396.

39. Rantala, M., and E. Nurmi. 1973. Prevention of the growth of *Salmonella infantis* in chicks by the flora of the alimentary tract of chickens. Br. Poult. Sci. 14:627–630. doi:10.1080/00071667308416073.

40. Blanchfield, B., S. Stavric, T. Gleeson, and H. Pivnick. 1984. Minimum intestinal inoculum for Nurmi cultures and a new method for determining competitive exclusion of *Salmonella* from chicks. J. Food Prot. 47:542–545. doi:10.4315/0362-028x-47.7.542.

41. Corrier, A. D. E., A. G. Hollister, D. J. Nisbet, C. M. Scanlan, and R. C. Beier. 1994. Competitive exclusion of *Salmonella enteritidis* in Leghorn Chicks: Comparison of treatment by crop gavage, drinking water, spray, or lyophilized alginate beads Avian Dis. 38:297–303

42. Kubasova, T., M. Kollarcikova, M. Crhanova, D. Karasova, D. Cejkova, A. Sebkova, J. Matiasovicova, M. Faldynova, F. Sisak, V. Babak, A. Pokorna, A. Cizek, and I. Rychlik. 2019. Gut anaerobes capable of chicken caecum colonisation. Microorganisms. 7:1–11. doi:10.3390/microorganisms7120597.

43. Schneitz, C. 2005. Competitive exclusion in poultry - 30 years of research. Food Control. 16:657-667. https://doi.org/10.1016/j.foodcont.2004.06.002.

44. Volf, J., O. Polansky, K. Varmuzova, L. Gerzova, Z. Sekelova, M. Faldynova, V. Babak, M. Medvecky, A. L. Smith, B. Kaspers, P. Velge, and I. Rychlik. 2016. Transient and prolonged response of chicken cecum mucosa to colonization with different gut microbiota. PLoS One. 11:1–18. doi:10.1371/journal.pone.0163932.

45. Meijerink, N., J. G. Kers, F. C. Velkers, D. A. van Haarlem, D. M. Lamot, J. E. de Oliveira, H. Smidt, J. A. Stegeman, V. P. M. G. Rutten, and C. A. Jansen. 2020. Early life inoculation with adult-derived microbiota accelerates maturation of intestinal microbiota and enhances NK cell activation in broiler chickens. Front. Vet. Sci. 7. doi:10.3389/fvets.2020.584561.

46. Yan, C., J. Xiao, Z. Li, H. Liu, Xinjie Zhao, J. Liu, S. Chen, and Xingbo Zhao. 2021. Exogenous fecal microbial transplantation alters fearfulness, intestinal morphology, and gut microbiota in broilers. Front. Vet. Sci. 8:1–11. doi:10.3389/fvets.2021.706987.

47. Zaytsoff et al., S. J. M., T. Montina, V. F. Boras, J. Brassard, P. E. Moote, R. R. E. Uwiera, and G. D. Inglis. 2022. Microbiota transplantation in day-old broiler chickens ameliorates necrotic enteritis via modulation of the intestinal microbiota and host immune responses. Pathogens. 11. doi:10.3390/pathogens11090972.

48. Glendinning, L., C. Chintoan-Uta, M. P. Stevens, and M. Watson. 2022. Effect of cecal microbiota transplantation between different broiler breeds on the chick flora in the first week of life. Poult. Sci. 101:101624. doi:10.1016/j.psj.2021.101624.

49. Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Stegeman JA, Smidt H. Host and Environmental Factors Affecting the Intestinal Microbiota in Chickens. Front Microbiol. 2018 Feb 16;9:235. doi: 10.3389/fmicb.2018.00235. PMID: 29503637; PMCID: PMC5820305.

50. Han Z, Sun J, Lv A, Wang A. 2019. Biases from different DNA extraction methods in intestine microbiome research based on 16S rDNA sequencing: a case in the koi carp, Cyprinus carpio var. Koi. Microbiologyopen. 8(1):e00626. doi: 10.1002/mbo3.626.

51. Brooks, J.P., Edwards, D.J., Harwich, M.D. et al. 2015. The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies. BMC Microbiol 15, 66 https://doi.org/10.1186/s12866-015-0351-6

52. Darwish, N., Shao, J., Schreier, L.L. et al. 2021. Choice of 16S ribosomal RNA primers affects the microbiome analysis in chicken ceca. Sci Rep 11, 11848. https://doi.org/10.1038/s41598-021-91387-w

53. López-García A, Pineda-Quiroga C, Atxaerandio R, Pérez A, Hernández I, García-Rodríguez A and González-Recio O. 2018. Comparison of Mothur and QIIME for the Analysis of Rumen Microbiota Composition Based on 16S rRNA Amplicon Sequences. Front. Microbiol. 9:3010. doi: 10.3389/fmicb.2018.03010

54. Nearing, J.T., Douglas, G.M., Hayes, M.G. et al. 2022. Microbiome differential abundance methods produce different results across 38 datasets. Nat Commun 13, 342. https://doi.org/10.1038/s41467-022-28034-

55. Stanley D, Geier MS, Hughes RJ, Denman SE, Moore RJ. 2013. Highly Variable Microbiota Development in the Chicken Gastrointestinal Tract. PLoS ONE 8(12): e84290. https://doi.org/10.1371/journal.pone.0084290

56. Jones, E. W., Carlson, J. M., Sivak, D. A., Ludington, W. B., 2022. Stochastic microbiome assembly depends on context. PNAS. 119 (7) e2115877119 https://doi.org/10.1073/pnas.2115877119

57. Gloor GB, Macklaim JM, Pawlowsky-Glahn V and Egozcue JJ. 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. Front. Microbiol. 8:2224. doi: 10.3389/fmicb.2017.02224

58. Ramírez, G. A., E. Richardson, J. Clark, J. Keshri, Y. Drechsler, M. E. Berrang, R. J. Meinersmann, N. A. Cox, and B. B. Oakley. 2020. Broiler chickens and early life programming: Microbiome transplant-induced cecal community dynamics and phenotypic effects. PLoS One. 15:1–22. doi:10.1371/journal.pone.0242108.

59. Gong Y, Yang H, Wang X, Xia W, Lv W, Xiao Y and Zou X. 2019. Early Intervention With Cecal Fermentation Broth Regulates the Colonization and Development of Gut Microbiota in Broiler Chickens. Front. Microbiol. 10:1422. doi: 10.3389/fmicb.2019.01422

60. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. 2019. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS ONE 14(3): e0212446. https://doi.org/10.1371/journal.pone.0212446

61. Barnes, E. M., C. S. Impey, and BJ.H. Stevens, 1979. Factors affecting the incidence and anti-*Salmonella* activity of the anaerobic caecal flora of the young chick. J. Hyg. Camb. 82:263-283. DOI: 10.1017/s0022172400025687

62. Scupham AJ, Jones JA, Rettedal EA, Weber TE. 2010. Antibiotic manipulation of intestinal microbiota to identify microbes associated with *Campylobacter jejuni* exclusion in poultry. Appl Environ Microbiol. 76(24):8026-32. doi: 10.1128/AEM.00678-10

63. Fan, Y., Ju, T., Bhardwaj, T., Korver, D. R, Willing, B. P. 2023 Week-old chicks with high *Bacteroides* abundance have increased short-chain fatty acids and reduced markers of gut inflammation. Microbiology Spectrum 10.1128/spectrum.03616-22 DOI: https://doi.org/10.1128/spectrum.03616-22

64. Bar Shira, E. and Friedman, A. 2018. Innate immune functions of avian intestinal epithelial cells: response to bacterial stimuli and localization of responding cells in the developing avian digestive tract. PLoS One 13(7): e0200393. https://doi.org/10.1371/journal.pone.0200393.

65. Waititu, S. M., A. Yitbarek, E. Matini, H. Echeverry, E. Kiarie, J. C. Rodriguez-Lecompte, and C. M. Nyachoti. 2014. Effect of supplementing direct-fed microbials on broiler performance, nutrient digestibilities, and immune responses. Poult. Sci. 93:625–635. doi:10.3382/ps.2013-03575.

66. Bäckhed, F., H. Ding, T. Wang, L. V. Hooper, Y. K. Gou, A. Nagy, C. F. Semenkovich, and J. I. Gordon. 2004. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. U. S. A. 101:15718–15723. doi:10.1073/pnas.0407076101.

67. Turnbaugh, P. J., R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis, and J. I. Gordon. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 444:1027–1031. doi:10.1038/nature05414.

68. Ley, R., Turnbaugh, P., Klein, S., Gordon, J. 2006. Human gut microbes associated with obesity. Nature 444:1022-1023. https://doi.org/10.1038/4441022a

69. Sapan, H. B., I. Paturusi, I. Jusuf, I. Patellongi, M. N. Massi, A. D. Pusponegoro, S. K. Arief, I. Labeda, A. A. Islam, L. Rendy, and M. Hatta. 2016. Pattern of cytokine (IL-6 and IL-10) level as inflammation and anti-inflammation mediator of multiple organ dysfunction syndrome (MODS) in polytrauma. Int. J. Burns Trauma. 6:37–43.

70. Samad, F., G. Bergtrom, H. Eissa, and D. L. Amrani. 1993. Stimulation of chick hepatocyte fibronectin production by fibroblast-conditioned medium is due to interleukin 6. BBA - Mol. Basis Dis. 1181:207–213. doi:10.1016/0925-4439(93)90022-S.

71. Marais, M., S. K. Maloney, and D. A. Gray. 2011. Brain IL-6- and PG-dependent actions of IL-1β and lipopolysaccharide in avian fever. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 301. doi:10.1152/ajpregu.00136.2011.

72. Prakasam, R., M. Fujimoto, R. Takii, N. Hayashida, E. Takaki, K. Tan, F. Wu, S. Inouye, and A. Nakai. 2013. Chicken IL-6 is a heat-shock gene. FEBS Lett. 587:3541–3547. doi:10.1016/j.febslet.2013.09.012.

73. Nishimichi, N., T. Kawashima, S. Hojyo, H. Horiuchi, S. Furusawa, and H. Matsuda. 2006. Characterization and expression analysis of a chicken interleukin-6 receptor alpha. Dev. Comp. Immunol. 30:419–429. doi:10.1016/j.dci.2005.05.007.

74. Schneider, K., R. Klaas, B. Kaspers, and P. Staeheli. 2001. Chicken interleukin-6: cDNA structure and biological properties. Eur. J. Biochem. 268:4200–4206. doi:10.1046/j.1432-1327.2001.02334.x.

75. Fasina, Y. O., Holt, P.S., Moran, E.T., Moore, R.W., Conner, D. E., McKee. S.R. 2008. Intestinal cytokine response of commercial source broiler chicks to *Salmonella Typhimurium* infection. Poult. Sci. 87:1335-1346. doi: https://doi.org/10.3382/ps.2007-00526.

76. Kaiser, P., L. Rothwell, E. E. Galyov, P. A. Barrow, J. Burnside, and P. Wigley. 2000. Differential cytokine expression in avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella gallinarum*. Microbiology. 146:3217–3226. doi:10.1099/00221287-146-12-3217.

77. Lynagh, G. R., M. Bailey, and P. Kaiser. 2000. Interleukin-6 is produced during both murine and avian *Eimeria* infections. Vet. Immunol. Immunopathol. 76:89–102. doi:10.1016/S0165-2427(00)00203-8.

78. Xie, H., N. C. Rath, G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Effects of *Salmonella typhimurium* lipopolysaccharide on broiler chickens. Poult. Sci. 79:33–40. doi:10.1093/ps/79.1.33.

79. Rothwell, L., J. R. Young, R. Zoorob, C. A. Whittaker, P. Hesketh, A. Archer, A. L. Smith, and P. Kaiser. 2004. Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. J. Immunol. 173:2675–2682. doi:10.4049/jimmunol.173.4.2675.

80. Park, S. S., Lillehoj, H. S., Allen, P. C., Park, D. W., FitzCoy, S., Bautista, D. A., & Lilleho, E. P. 2008. Immunopathology and Cytokine Responses in Broiler Chickens Coinfected with *Eimeria Maxima* and *Clostridium perfringens* with the Use of an Animal Model of Necrotic Enteritis. Avian Diseases, 52(1), 14–22. http://www.jstor.org/stable/27565692

81. Tang, Y., N. Foster, M. A. Jones, and P. A. Barrow. 2018. Model of persistent *Salmonella* infection: *Salmonella enterica* serovar *Pullorum* modulates the immune response of the chicken from a Th17-type response towards a Th2-type response Response. Infect. Immun. 86:1–20. doi:10.1128/IAI.00307-18.

82. Ferro PJ, Swaggerty CL, Kaiser P, Pevzner IY, Kogut MH. 2004. Heterophils isolated from chickens resistant to extra-intestinal *Salmonella enteritidis* infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from susceptible chickens. Epidemiol Infect. 132(6):1029-37. doi: 10.1017/s0950268804002687.

83. Liu, Z., L. Wang, Y. Yu, A. Fotin, Q. Wang, P. Gao, Y. Zhang, T. Fotina, and J. Ma. 2022. SteE Enhances the virulence of *Salmonella Pullorum* in chickens by regulating the inflammation response. Front. Vet. Sci. 9:1–12. doi:10.3389/fvets.2022.926505.

84. Ghebremicael, S. B., J. R. Hasenstein, and S. J. Lamont. 2008. Association of interleukin-10 cluster genes and *Salmonella* response in the chicken. Poult. Sci. 87:22–26. doi:10.3382/ps.2007-00259.

85. Kogut, M.H.; Swaggerty, C.L.; Byrd, J.A.; Selvaraj, R.; Arsenault, R.J. 2016. Chicken-Specific Kinome Array Reveals that Salmonella enterica Serovar Enteritidis Modulates Host Immune Signaling Pathways in the Cecum to Establish a Persistence Infection. Int. J. Mol. Sci. 2016, 17, 1207. https://doi.org/10.3390/ijms17081207

86. Rose-John, S., K. Winthrop, and L. Calabrese. 2017. The role of IL-6 in host defence against infections: Immunobiology and clinical implications. Nat. Rev. Rheumatol. 13:399–409. doi:10.1038/nrrheum.2017.83.

87. Rakoff-Nahoum, S., J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov. 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 118:229–241. doi:10.1016/j.cell.2004.07.002.

88. Kuhn, K. A., H. M. Schulz, E. H. Regner, E. L. Severs, J. D. Hendrickson, G. Mehta, A. K. Whitney, D. Ir, N. Ohri, C. E. Robertson, D. N. Frank, E. L. Campbell, and S. P. Colgan. 2018. Bacteroidales recruit IL-6-producing intraepithelial lymphocytes in the colon to promote barrier integrity. Mucosal Immunol. 11:357–368. doi:10.1038/mi.2017.55.

89. Zenner, C., T. C. A. Hitch, T. Riedel, E. Wortmann, S. Tiede, E. M. Buhl, and B. Abt. 2021. Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. mSystems. 6:e01300-20. https://doi.org/10.1128/mSystems.01300-20.

90. Fu, Y., J. Hu, M. A. Erasmus, T. A. Johnson, and H. wei Cheng. 2022. Effects of early-life cecal microbiota transplantation from divergently selected inbred chicken lines on growth, gut

serotonin, and immune parameters in recipient chickens. Poult. Sci. 101:1–11. doi:10.1016/j.psj.2022.101925.

91. Li, P., M. Gao, B. Song, Y. Liu, S. Yan, J. Lei, Y. Zhao, G. Li, T. Mahmood, Z. Lv, Y. Hu, and Y. Guo. 2022. Fecal microbiota transplantation reshapes the physiological function of the intestine in antibiotic-treated specific pathogen-free birds. Front. Immunol. 13:1–13. doi:10.3389/fimmu.2022.884615.

92. Hu, J., Yu, H., Kulkarni, R. R., Sharif, S., Cui, S.W., Xie M. Y., Nie, S. P., Gong J. 2015 Modulation of cytokine gene expression by selected *Lactobacillus* isolates in the ileum, caecal tonsils and spleen of *Salmonella*-challenged broilers, Avian Pathology, 44:6, 463-469, DOI: 10.1080/03079457.2015.1086725

93. Hong, Y. H., H. S. Lillehoj, E. P. Lillehoj, and S. H. Lee. 2006. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following *Eimeria maxima* infection of chickens. Vet. Immunol. Immunopathol. 114:259–272. doi:10.1016/j.vetimm.2006.08.006.

94. Coble DJ, Redmond SB, Hale B, Lamont SJ. 2011. Distinct lines of chickens express different splenic cytokine profiles in response to *Salmonella* Enteritidis challenge. Poult Sci. (8):1659-63. doi: 10.3382/ps.2010-01279.

95. Hume, M. E., D. E. Corrier, S. Ambrus, A. Hinton, and J. R. DeLoach. 1993. Effectiveness of dietary propionic acid in controlling *Salmonella typhimurium* colonization in broiler chicks. Avian Dis. 37:1051–1056. doi:10.2307/1591912.

96. Nisbet, D. J., D. E. Corrier, S. C. Ricke, M. E. Hume, J. A. Byrd, and J. R. DeLoach. 1996. Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to *Salmonella* in chicks. Anaerobe. 2:345–350. doi:10.1006/anae.1996.0044.

97. Elsherif HMR, Orabi A, Hassan HMA, Samy A. 2022. Sodium formate, acetate, and propionate as effective feed additives in broiler diets to enhance productive performance, blood biochemical, immunological status and gut integrity. Adv. Anim. Vet. Sci. 10(6):1414-1422. https://dx.doi.org/10.17582/journal.aavs/2022/10.6.1414.1422

98. Polansky, O., Z. Sekelova, M. Faldynova, A. Sebkova, F. Sisak, I. Rychlik, L. 2016. Important metabolic pathways and biological processes expressed by chicken cecal Microbiota. 82:1569–1576. doi: 10.1128/AEM.03473-15

99. Luo, J., C. S. Ranadheera, S. King, C. A. Evans, and S. Baines. 2018. Dairy propionibacteria as direct-fed microbials: in vitro effect on acid metabolism of Streptococcus bovis and Megasphaera elsdenii. Ann. Microbiol. 68:153–158. doi:10.1007/s13213-018-1322-6.

100. Shetty SA, Marathe NP, Lanjekar V, Ranade D, Shouche YS. 2013. Comparative genome analysis of *Megasphaera* sp. reveals niche specialization and its potential role in the human gut. PLoS One. 2013 Nov 18;8(11):e79353. doi: 10.1371/journal.pone.0079353

101. Maki JJ, Looft T. 2018. Megasphaera stantonii sp. nov., a butyrate-producing bacterium isolated from the cecum of a healthy chicken. Int J Syst Evol Microbiol. 68(11):3409-3415. doi: 10.1099/ijsem.0.002991.

102. Onrust, L., K. Van Driessche, R. Ducatelle, K. Schwarzer, F. Haesebrouck, and F. Van Immerseel. 2018. Valeric acid glyceride esters in feed promote broiler performance and reduce the incidence of necrotic enteritis. Poult. Sci. 97:2303–2311. doi:10.3382/ps/pey085.

103. Iino, T., K. Mori, K. Tanaka, K. I. Suzuki, and S. Harayama. 2007. *Oscillibacter valericigenes* gen. nov., sp. nov., a valerate-producing anaerobic bacterium isolated from the alimentary canal of a Japanese corbicula clam. Int. J. Syst. Evol. Microbiol. 57:1840–1845. doi:10.1099/ijs.0.64717-0.

104. Yoshikawa, S., R. Araoka, Y. Kajihara, T. Ito, H. Miyamoto, and H. Kodama. 2018. Valerate production by *Megasphaera elsdenii* isolated from pig feces. J. Biosci. Bioeng. 125:519–524. doi:10.1016/j.jbiosc.2017.12.016.

105. Berg, G., D. Rybakova, D. Fischer, T. Cernava, M.-C. C. Vergès, T. Charles, X. Chen, L. Cocolin, K. Eversole, G. H. Corral, M. Kazou, L. Kinkel, L. Lange, N. Lima, A. Loy, J. A. Macklin, E. Maguin, T. Mauchline, R. McClure, B. Mitter, M. Ryan, I. Sarand, H. Smidt, B. Schelkle, H. Roume, G. S. Kiran, J. Selvin, R. S. C. de Souza, L. van Overbeek, B. K. Singh, M. Wagner, A. Walsh, A. Sessitsch, and M. Schloter. 2020. Correction to: Microbiome definition re-visited: old concepts and new challenges. Microbiome. 8:1–22. doi:10.1186/s40168-020-00905-x.

106. Neu, A. T. 2021. Defining and quantifying the core microbiome : Challenges and prospects. 118:1–10. doi:10.1073/pnas.2104429118/-/DCSupplemental.Published.

107. Darnaud, M., F. De Vadder, P. Bogeat, L. Boucinha, A. L. Bulteau, A. Bunescu, C. Couturier, A. Delgado, H. Dugua, C. Elie, A. Mathieu, T. Novotná, D. A. Ouattara, S. Planel, A. Saliou, D. Šrůtková, J. Yansouni, B. Stecher, M. Schwarzer, F. Leulier, and A. Tamellini. 2021. A standardized gnotobiotic mouse model harboring a minimal 15-member mouse gut microbiota recapitulates SOPF/SPF phenotypes. Nat. Commun. 12:1–21. doi:10.1038/s41467-021-26963-9.

108. Kollarcikova M, Faldynova M, Matiasovicova J, Jahodarova E, Kubasova T, Seidlerova Z, Babak V, Videnska P, Cizek A, Rychlik I. 2020. Different *Bacteroides* Species Colonise Human and Chicken Intestinal Tract. Microorganisms. 8(10):1483. doi: 10.3390/microorganisms8101483.

109. Poudel, B., N. Shterzer, Y. Sbehat, N. Ben-Porat, M. Rakover, R. Tovy-Sharon, D. Wolicki, S. Rahamim, E. Bar-Shira, and E. Mills. 2022. Characterizing the chicken gut colonization ability of a diverse group of bacteria. Poult. Sci. 101:102136. doi:10.1016/j.psj.2022.102136.

110. Marcolla, C.S., Alvarado, C.S., and Willing, B. P. 2019. Early life microbial exposure shapes subsequent animal health. Canadian Journal of Animal Science. 99(4): 661-677. https://doi.org/10.1139/cjas-2019-0029

111. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, Hickman HD, McCulloch JA, Badger JH, Ajami NJ, Trinchieri G, Pardo-Manuel de Villena F, Yewdell JW, Rehermann B. 2017. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell.; 171(5):1015-1028.e13. doi: 10.1016/j.cell.2017.09.016.

112. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, McCulloch JA, Anastasakis DG, Sarshad AA, Leonardi I, Collins N, Blatter JA, Han SJ, Tamoutounour S, Potapova S, Foster St Claire MB, Yuan W, Sen SK, Dreier MS, Hild B, Hafner M, Wang D, Iliev ID, Belkaid Y, Trinchieri G, Rehermann B. 2019. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science. 365(6452):eaaw4361. doi: 10.1126/science.aaw4361.

113. Souza MR, Moreira JL, Barbosa FH, Cerqueira MM, Nunes AC, Nicoli JR. 2007. Influence of intensive and extensive breeding on lactic acid bacteria isolated from *Gallus gallus domesticus* ceca. Vet Microbiol. 120(1-2):142-50. doi: 10.1016/j.vetmic.2006.10.019.

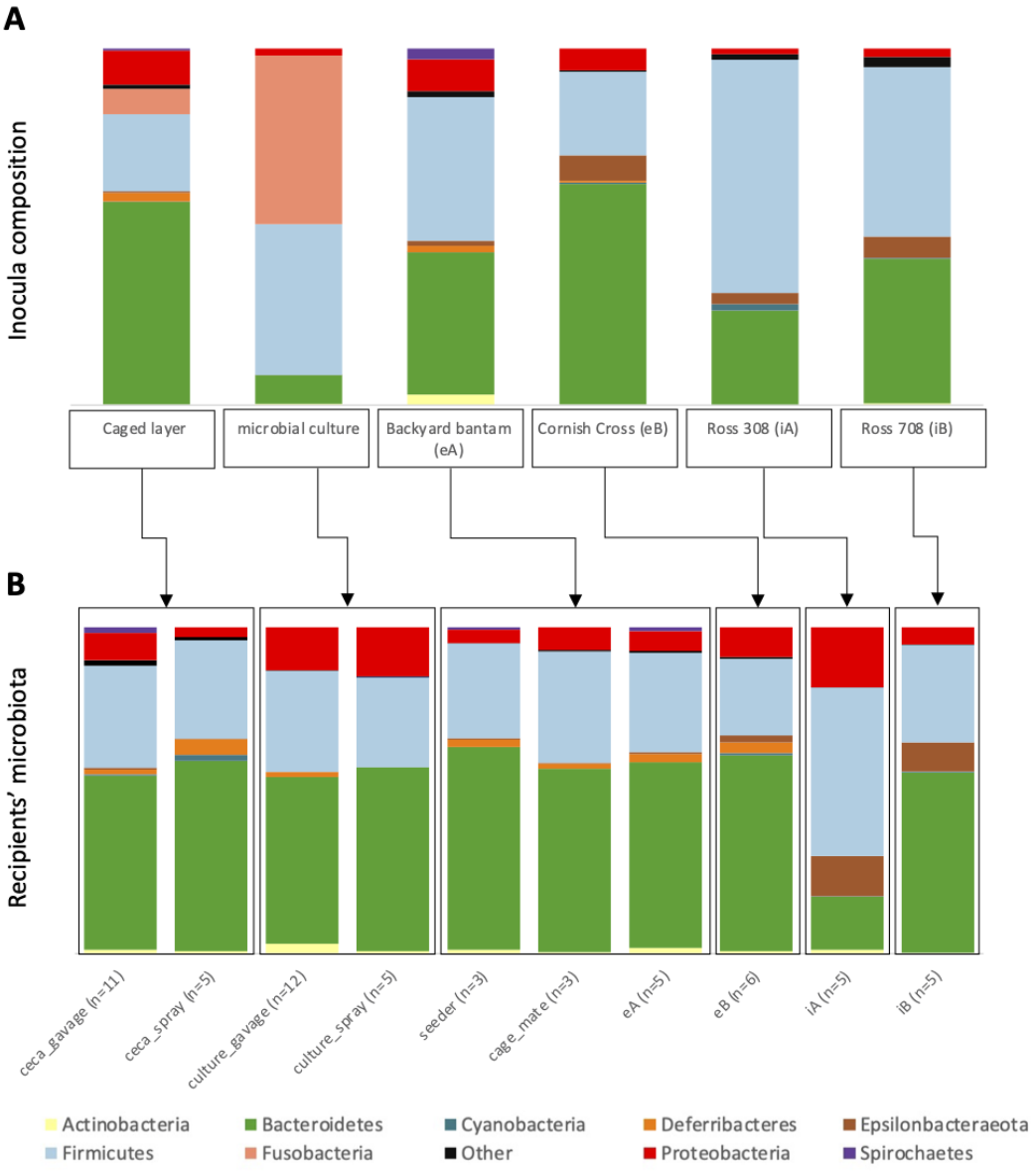


Figure 4.1. Bar plots showing (A) the abundance of phyla in inocula introduced to day-old chicks and (B) the average relative abundance of phyla in the cecal microbiota of recipient birds.

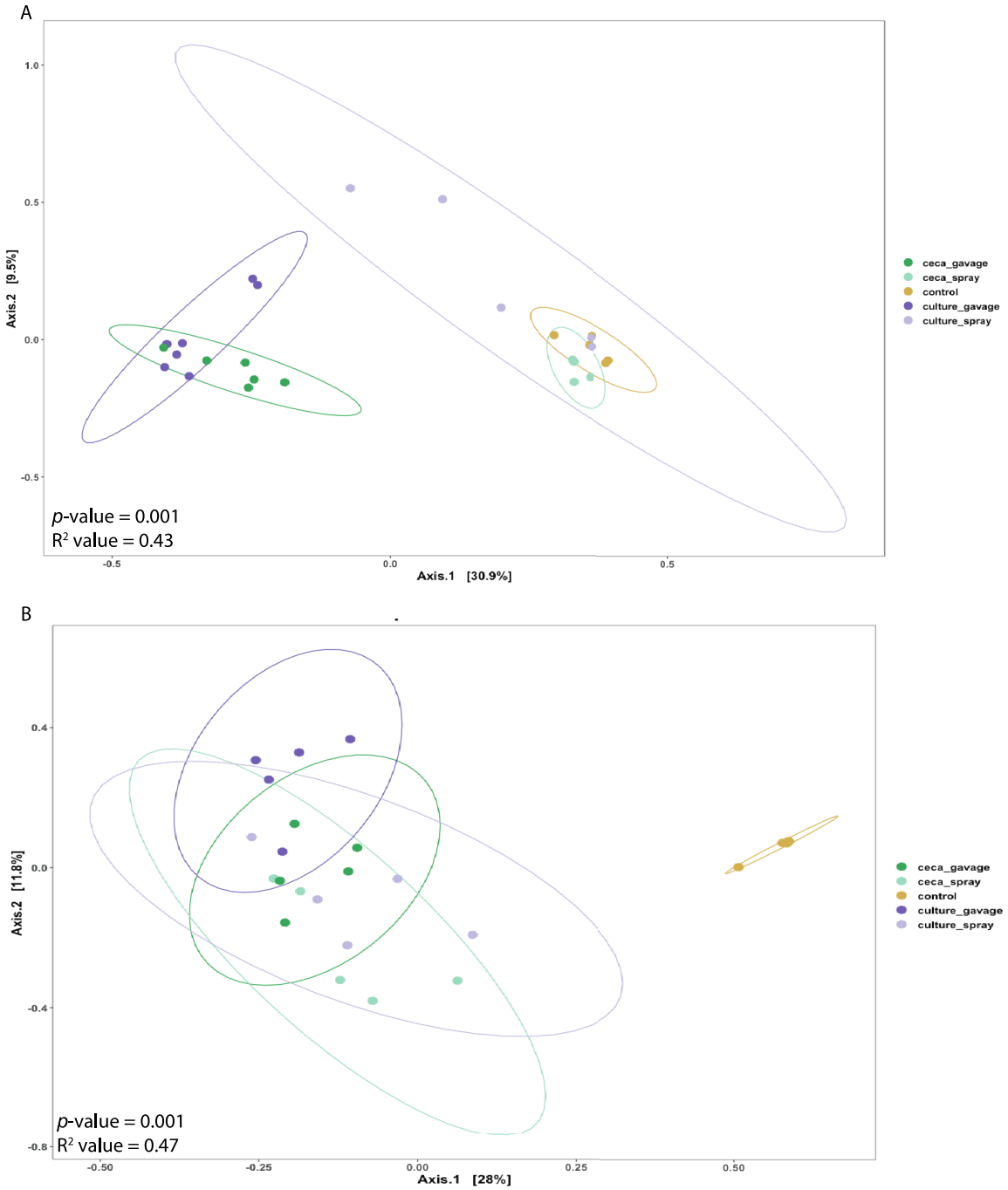


Figure 4.2. Principal coordinates analysis (PCoA) generated based on Bray-Curtis dissimilarity of cecal samples obtained from 21-day-old broilers in the inoculation strategies experiment including trials A (A) and B (B). Samples are colored and shaped according to treatments and

data ellipses represent the 95% confidence region for group clusters assuming a multivariate t-distribution.

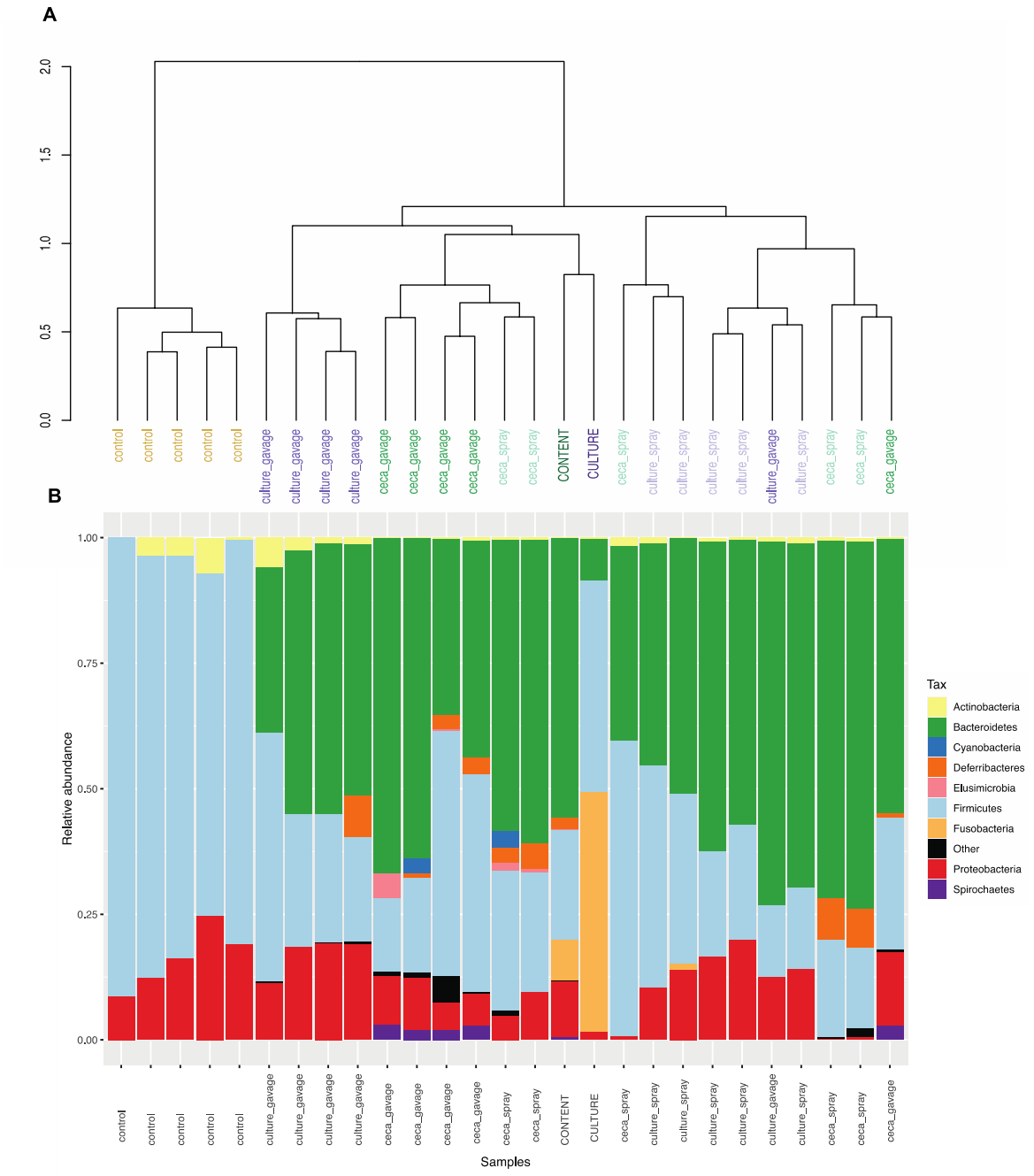
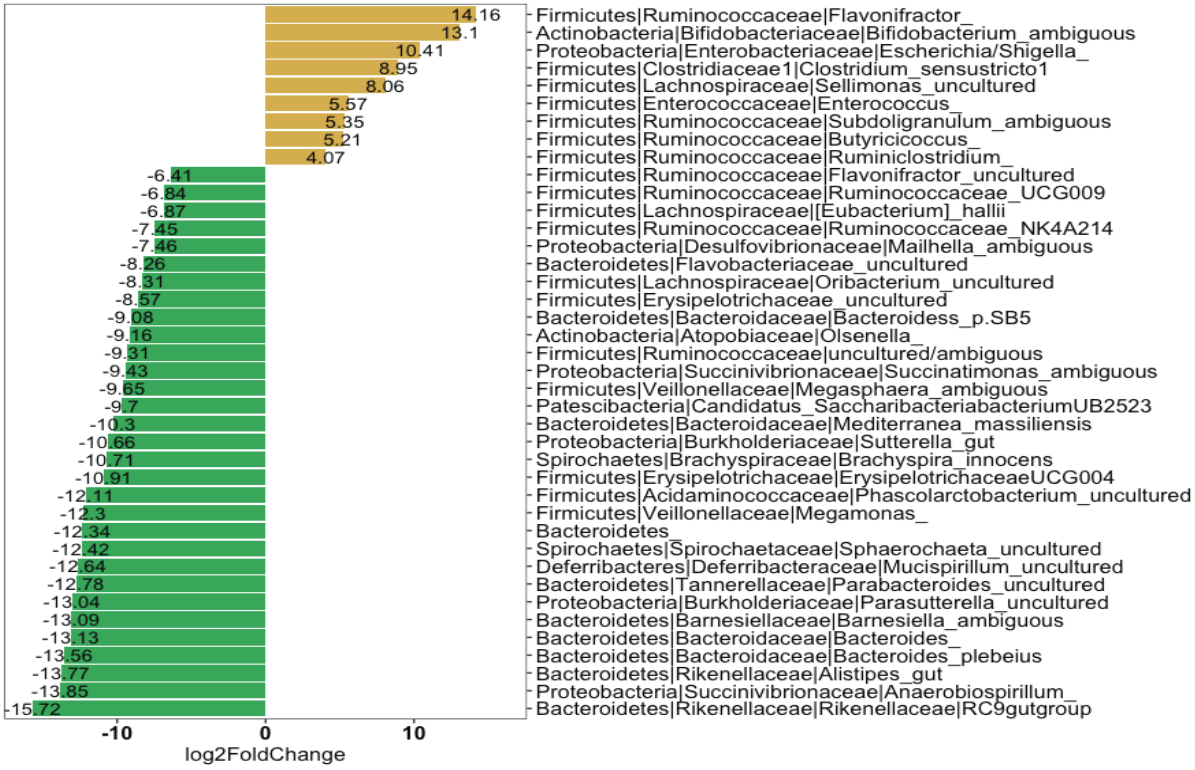


Figure 4.3. (A) Dendrogram showing hierarchical clustering based on Bray-Curtis matrices and (B) Bar plots showing the relative abundance of phyla in cecal samples obtained from 21-day-old broilers in inoculation strategies experiment (trial B) and inocula ("CONTENT"= ceca content; "CULTURE" = microbial culture). Phyla observed in less than 5% of samples and that had less than 1% relative abundance were combined as "Other" (black).

A Log2 Fold-Change for control vs. ceca_gavage - trial B



B Log2 Fold-Change for control vs. culture_gavage - trial B

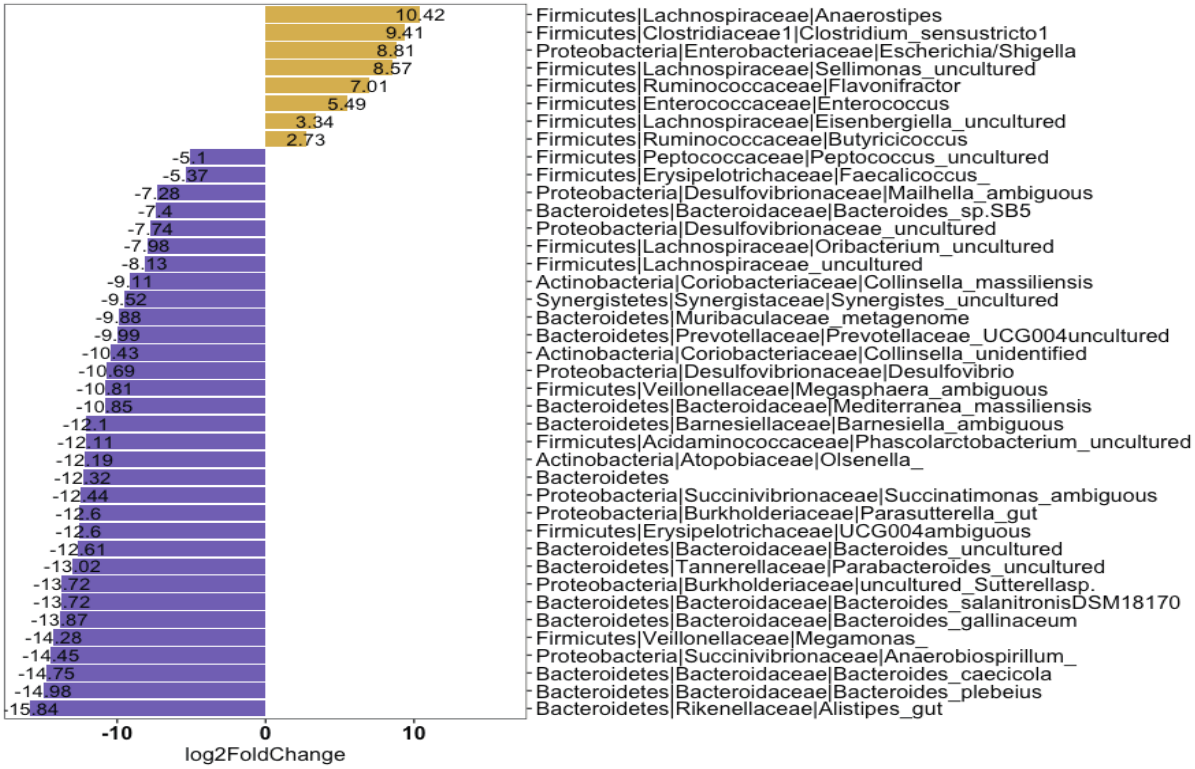
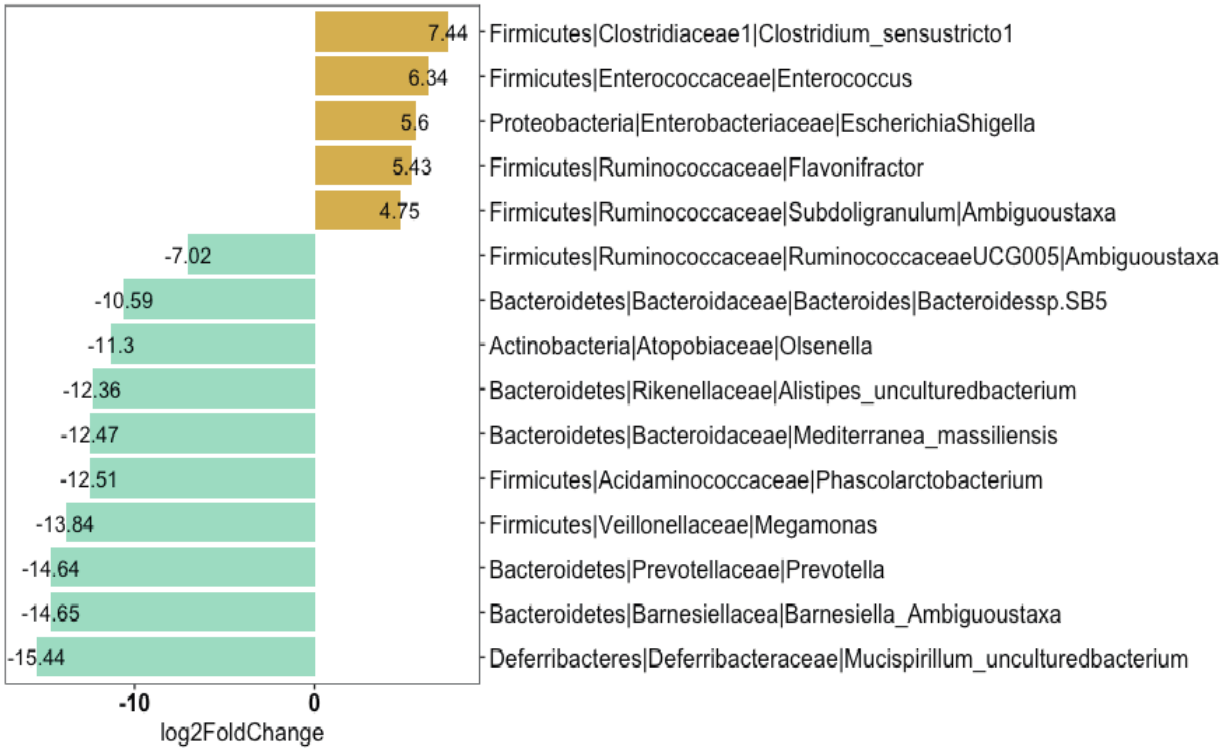


Figure 4.4. (A) Bar plots showing $\log_2$ fold change values of taxa enriched in control birds (yellow, positive values, n = 5) and ceca_gavage birds (dark green, negative values, n = 5); and (B) control birds (yellow, positive values) and culture_gavage birds (dark purple, negative values, n = 5) in trial B of the inoculation strategies experiment.

A Log2 Fold-Change for control vs. ceca_spray - trial B



B Log2 Fold-Change for control vs. culture_spray - trial B

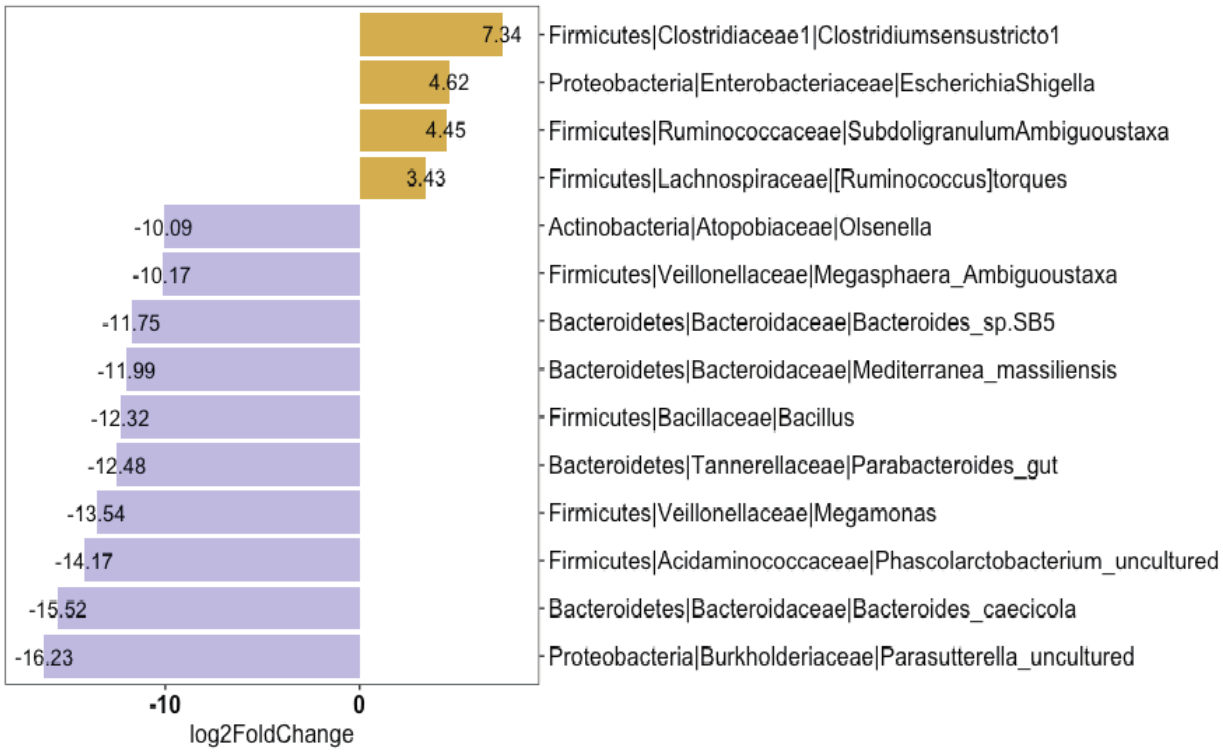


Figure 4.5. Bar plots showing log_2 fold change values of taxa enriched in **(A)** control birds (yellow, positive values, n = 5) and ceca_spray birds (light green, negative values, n = 5), and in **(B)** control birds (yellow, positive values) and culture_spray (light purple, negative values, n = 5) birds in trial B of inoculation strategies experiment.

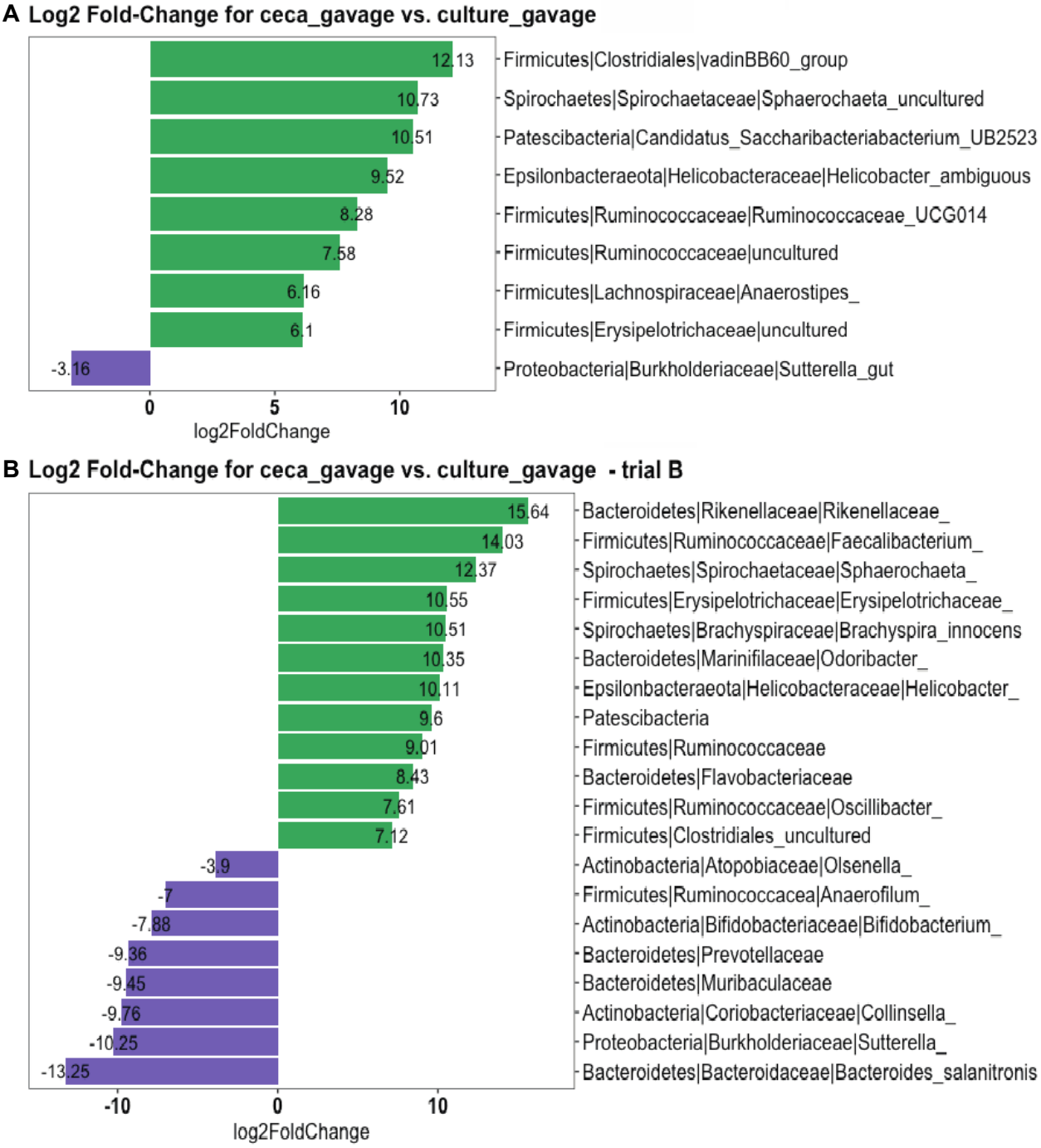


Figure 4.6. Bar plots showing $\log_2$ fold change values of taxa enriched in ceca_gavage (dark green, positive values, n = 5) compared to culture_gavage (dark purple, negative values, n = 7 in trial A and n = 5 in trial B) in trials A **(A)** and B **(B)**.

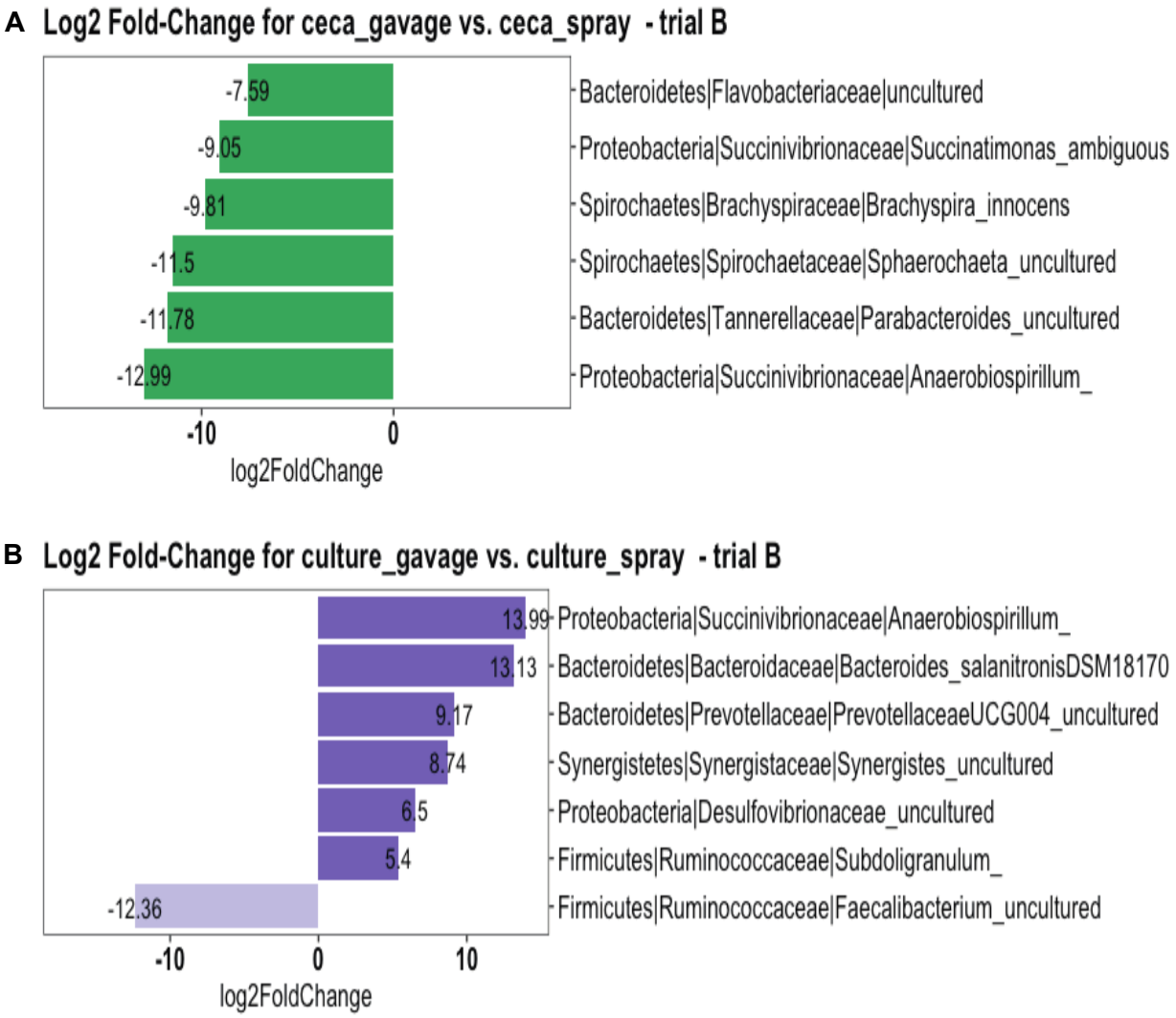


Figure 4.7. Bar plots showing $\log_2$ fold change values of taxa enriched in **(A)** ceca_gavage (dark green, negative values, n = 5) compared to ceca_spray (no enriched taxa detected, n = 5) and **(B)** culture_gavage (dark purple, positive values, n = 5) compared to culture_spray (light purple, negative values, n = 5) in trial B of inoculation strategies experiment.

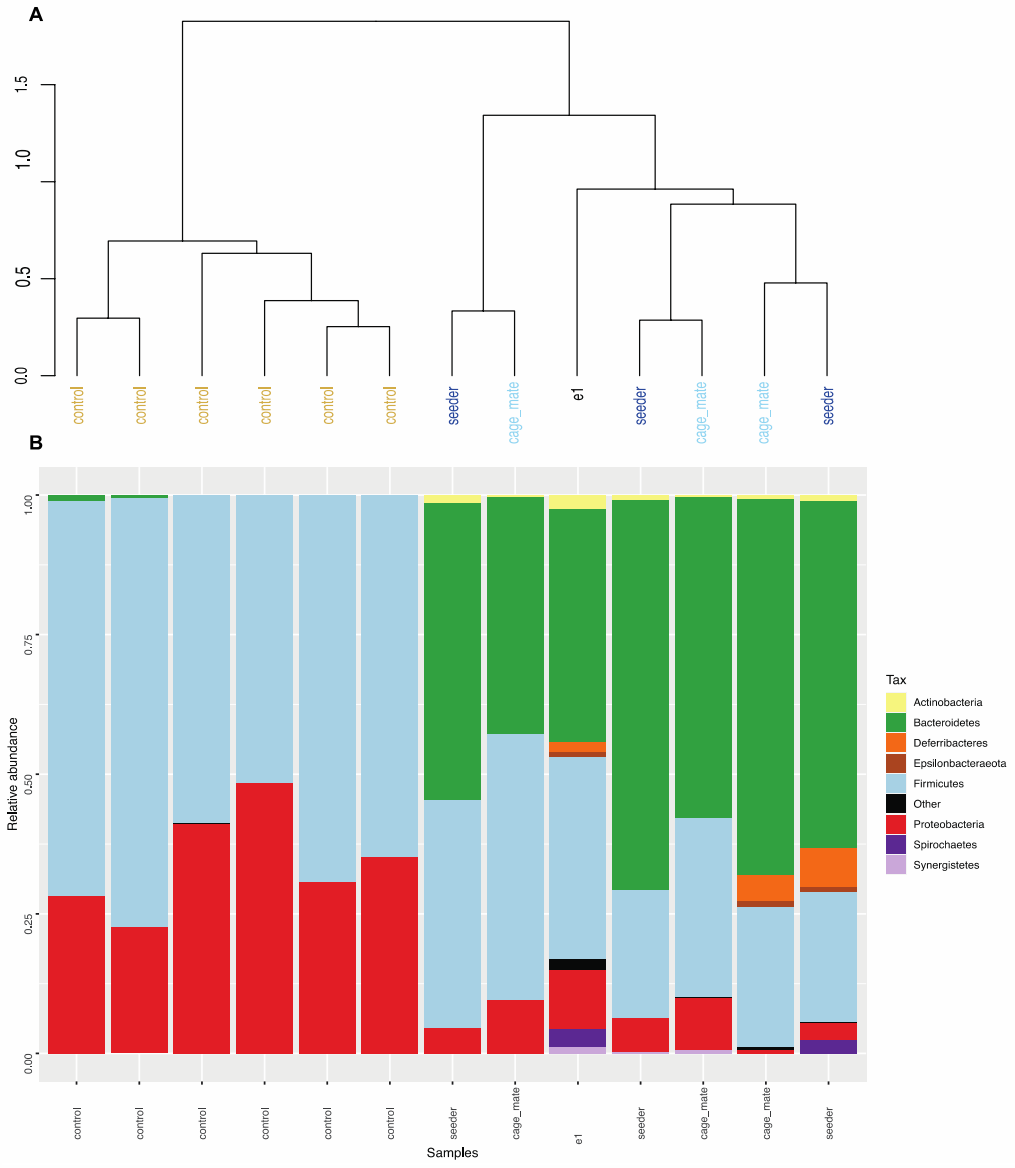


Figure 4.8. (A) Dendrogram showing hierarchical clustering based on Bray-Curtis metrics and **(B)** bar plots showing the relative abundance of phyla in cecal samples obtained from 14-day old broilers in the cohousing experiment. Phyla observed in less than 5% of samples and with less than 1% relative abundance were combined as "Other" (black).

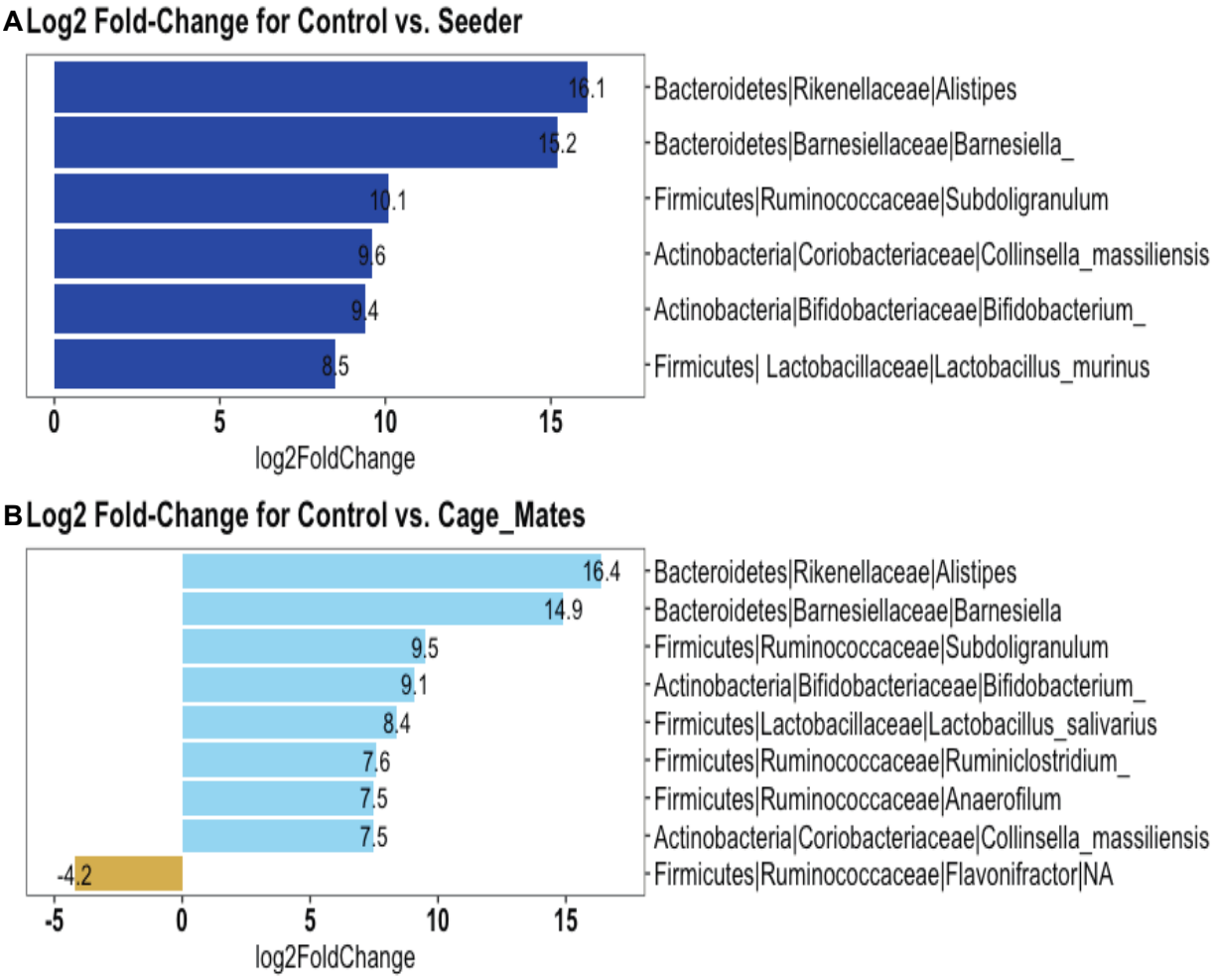


Figure 4.9. Bar plots showing $\log_2$ fold change values of taxa enriched in **(A)** seeder birds (navy blue, positive values, n = 3) and in **(B)** cage mates (light blue, positive values, n = 3) compared to birds in the control treatment (yellow, negative values, n = 6). No taxa were found to be enriched in control birds when compared to seeder birds. Comparison between seeder birds and cage mates indicate that no taxa was found to be differentially abundant.

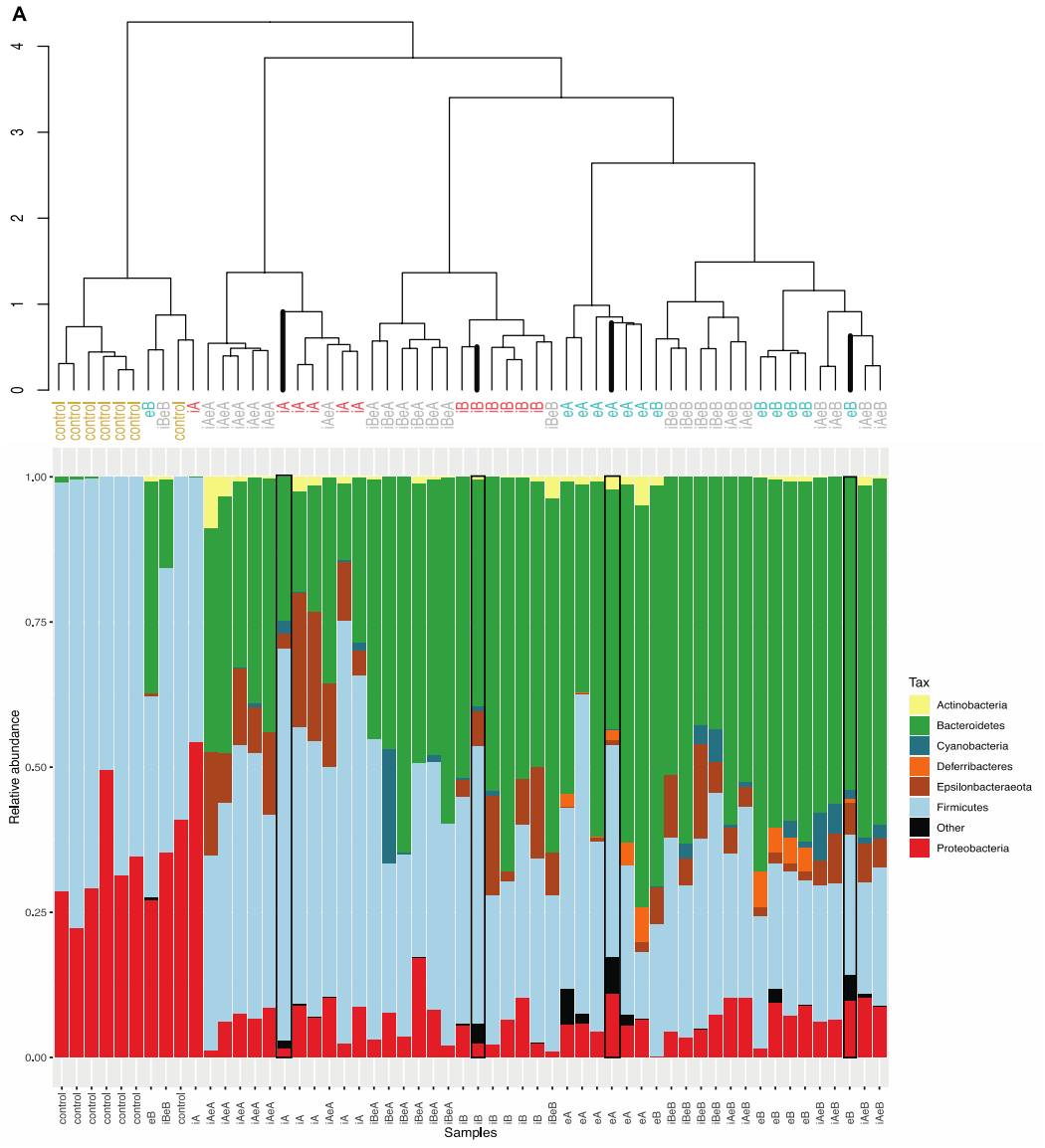


Figure 4.10. (A) Dendrogram showing hierarchical clustering based on Bray-Curtis matrices and **(B)** bar plots showing the relative abundance of phyla in cecal samples obtained from 14-day-old broilers in the competition experiment. Phyla observed in less than 5% of samples and with less than 1% relative abundance were combined as "Other" (black). Inocula are highlighted by the bold branches in the dendrogram and by the black boxes in the bar plots.

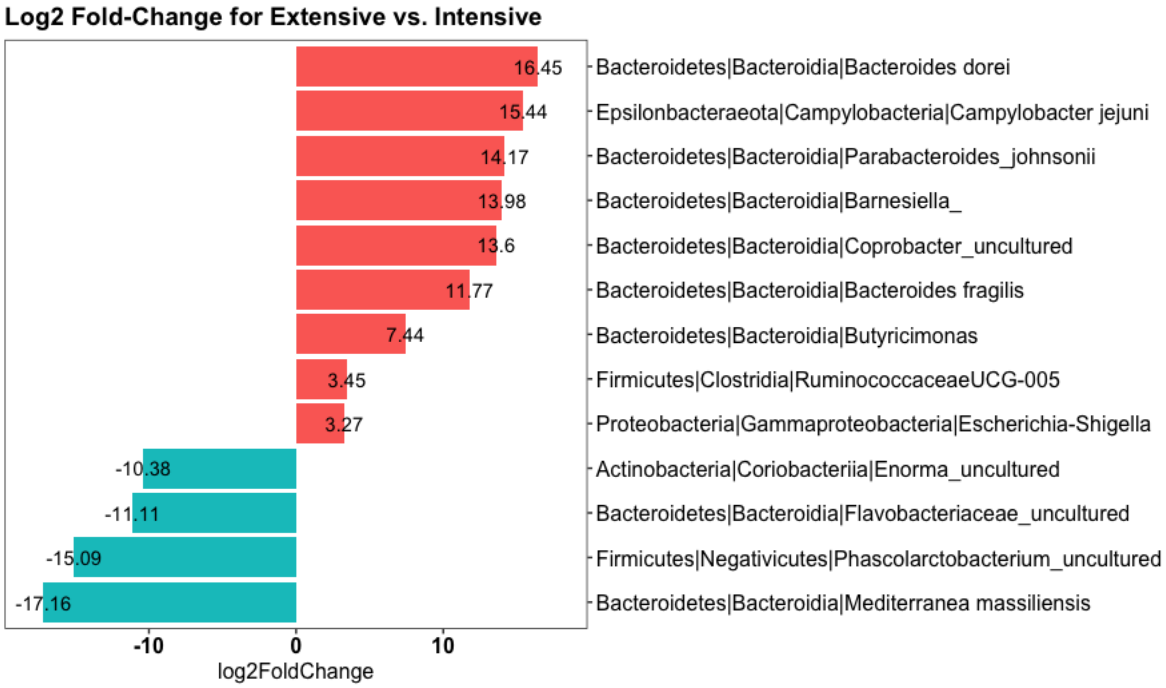


Figure 4.11. Bar plot showing $\log_2$ fold change values of taxa enriched in cecal contents of 14-day-old birds inoculated with cecal contents obtained from intensively raised broilers (red, positive values, n = 10) and birds inoculated with cecal contents obtained from extensively raised chickens (teal, negative values, n = 11).

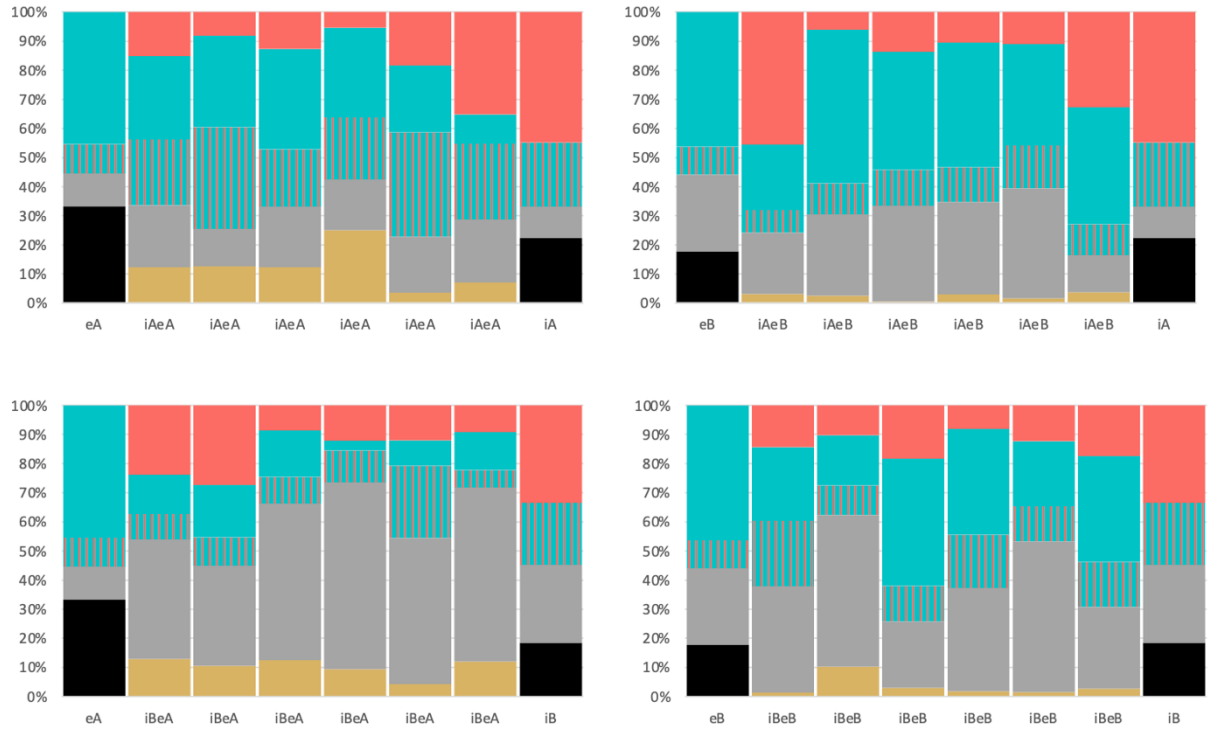


Figure 4.12. Simplified taxa plots showing the relative abundance of ASVs in the cecal samples and inocula of birds receiving mixed inocula in the competition experiment. ASV that were found exclusively in control birds are shown in yellow and represent the baseline microbiota, ASVs shared between inocula and control birds are shown in grey, ASVs shared between inocula from extensive and intensive systems are indicated by the striped pattern, ASVs unique to extensive inoculum are shown in teal, and ASVs unique to intensive inoculum are shown in red. ASVs that were present in the inocula and were absent in the chicken gut are shown in black.

Table 4.1. Effect of inoculation treatments on ileum morphology for 21-day-old broilers in trial B of inoculation strategies experiment

Treatment	VH[1]	SEM	CD[2]	SEM	VW[3]	SEM	VH/CD[4]	SEM
control	756.22[b]	16.03	112.37[b]	5.66	111.09[b]	9.87	6.94[a]	0.30
ceca_gavage	943.82[a]	38.44	165.27[a]	8.92	143.80[a]	9.36	5.95[ab]	0.33
ceca_spray	781.39[ab]	6.73	166.65[a]	6.42	152.48[a]	8.77	4.86[b]	0.21
culture_gavage	841.47[ab]	14.72	158.68[ab]	15.52	154.69[a]	10.15	6.07[ab]	0.71
culture_spray	705.90[b]	26.58	114.73[b]	4.69	130.76[ab]	9.41	6.40[a]	0.41

[a-b] Means within each column with no common superscript are significantly different after pairwise comparison using Tukey HSD test or Wilcoxon rank sum test with FDR correction. $\alpha = 0.05$. [1]VH = Villus height (μm); [2]CD = crypt depth (μm); [3]VW = villus width; [4]VH/CD = villus height to crypt depth ratio; SEM = standard error of the mean. Samples were collected from 5 birds in each treatment.

Table 4.2. Effect of inoculation treatments on the concentration (ng/g protein) of IL-6, IL-10 and VEGF in the ceca of 21-day-old broilers in trial B of inoculation strategies experiment.

Treatment	IL-6	SEM	IL-10	SEM	VEGF	SEM
control	159.88[b]	55.79	17.22[c]	5.14	44.48[ab]	12.58
ceca_gavage	301.12[a]	27.71	50.52[a]	11.06	46.86[ab]	4.68
ceca_spray	236.07[ab]	7.36	26.94[bc]	4.67	52.93[a]	3.97
culture_gavage	318.39[a]	34.61	39.14[abc]	7.5	32.38[ab]	9.18
culture_spray	348.94[a]	30.39	48.03[ab]	6.11	25.04[a]	9.86

[a-c]Means within each column with no common superscript are significantly different after pairwise comparison using Tukey HSD test or Wilcoxon rank sum test with FDR correction. $\alpha = 0.05$. Samples were collected from 5 birds in each treatment.

Table 4.3. Concentration of SCFA (µmol/g ceca content) in the cecal contents from 21-day-old broilers (n = 5 per treament) in trial B of inoculation strategies experiment.

Treatment	Acetate	SEM	Propionate	SEM	Isobutyrate	SEM	Butyrate	SEM	Isovalerate	SEM	Valerate	SEM
control	41.98	5.31	4.01[b]	0.35	0.16	0.04	3.82	1.02	0.09	0.03	0.06[c]	0.03
ceca_gavage	31.02	5.85	14.13[ab]	1.43	0.25	0.06	4.3	1.04	0.14	0.04	0.57[ab]	0.03
ceca_spray	40.53	5.81	11.93[a]	1.75	0.13	0.06	5.75	1.09	0.08	0.03	0.59[a]	0.02
culture_gavage	28.63	6.18	13.07[a]	1.75	0.23	0.05	2.58	1.06	0.1	0.02	0.13[c]	0.01
culture_spray	25.56	2.4	9.31[a]	1.38	0.38	0.06	2.51	0.34	0.23	0.02	0.27[b]	0.01

[a-c]Means within each column with no common superscript are significantly different after pairwise comparison using Tukey HSD test or Wilcoxon rank sum test with FDR correction. $\alpha = 0.05$

CHAPTER 5. Impact of a defined bacterial community and *Megamonas hypermegale* on broiler cecal microbiota and resistance to *Salmonella* infection

5.1 INTRODUCTION

Current poultry production practices aim to minimize bird exposure to pathogens that can cause disease and contaminate food products. These practices may impair the colonization of the chicken gastrointestinal tract with host-adapted commensal bacteria that might have co-evolved with chickens in nature [1–4]. In a previous study, we found that the gut microbiota of broilers reared in intensive systems was less diverse and depleted of non-spore-forming strict anaerobic bacteria, compared to that of age-matched broilers from extensive systems [4]. Moreover, other studies had demonstrated that intensively raised broilers lack bacterial species that are present in extensively raised birds and are effective colonizers of broiler ceca [4–7].

In our previous studies, we identified *Olsenella*, *Alistipes*, *Phocaeicola*, *Bacteroides*, *Barnesiella*, *Parabacteroides, Megamonas*, and *Parasutterella* as core bacterial genera within the broiler cecal microbiota that seem to be depleted in broilers in intensive systems [4]. Additionally, we demonstrated that chicks inoculated with cecal contents and undefined cecal cultures were consistently colonized by *Alistipes*, *Phocaeicola/Bacteroides*, *Barnesiella*, *Mediterranea*, *Megamonas*, *Parabacteroides*, *Phascolarctobacterium*, and *Subdoligranulum*, indicating that these bacteria are highly adapted and able to colonize the chicken gut after a single exposure [8].

Species from the *Megamonas* genus, including *Megamonas rupellensis*, *Megamonas funiformis*, and *Megamonas hypermegale*, have been isolated from chickens [9–11], and were enriched in the gut microbiota of wild and free-range birds [7, 12–14]. *M. hypermegale* is an anaerobic, Gram-negative, non-spore-forming, and non-motile rod that was first isolated from turkey feces [15]. *M. hypermegale* was shown to produce acetic, propionic, lactic and trace

amounts of succinic acids in broth culture [15, 16]. Metagenomics analysis indicated that *M. hypermegale* can metabolize hydrogen, potentially reducing the accumulation of H_2 that can hinder short-chain fatty acid production within the gut [17]. *M. hypermegale* cultured for 3 or 7 days in a high glucose media was demonstrated to reduce *in vitro* growth of *Salmonella* Typhimurium; however, in chicks inoculated with a 24 h culture of *M. hypermegale* at hatch and challenged with *S.* Typhimurium the next day, no inhibition of *Salmonella* load was observed [16]. Nonetheless, chicks inoculated with a defined microbial community containing *Phocaeicola vulgatus* (formerly *Bacteroides vulgatus*), *M. hypermegale* and another 46 bacterial isolates were shown to help with host resistance to *S.* Typhimurium infection [18]. Subsequently, when chicks were inoculated with the same defined microbial community and received a diet containing antibiotics, no inhibitory effect of these isolates on *Salmonella* levels was observed in the context of a depletion of *P. vulgatus* and *M. hypermegale*, suggesting that these species may play a key role in host resistance to *Salmonella* infection [18].

In addition, associations between *M. hypermegale* and other host health outcomes have been reported. For example, *M. hypermegale* abundance has been negatively associated with *Campylobacter jejuni* load in the turkey gut [19], and *M. hypermegale* abundance was increased in broilers that received cecal microbiota transplant and were challenged with *Clostridium perfringens* [20]. Despite its suggested importance as a core member of the chicken gut microbiome, the capability of *M. hypermegale* to colonize the chicken gut, particularly in early-life stage, and its potential role in inhibiting pathogenic infection have not been investigated. Therefore, this study aimed to evaluate the effect of early-life introduction of *M. hypermegale* alone or in combination with a defined community (DC) of bacteria on broiler gut microbiota development and host ability to resist *Salmonella* infection.

5.2 MATERIALS AND METHODS

The animals used in this study were housed and maintained according to requirements of Canadian Council on Animal Care and Canadian Biosafety Standards for Facilities Handling or Storing Human and Terrestrial Animal Pathogens and Toxins. This study was approved by the University of Alberta Animal Care and Use Committee (AUP00002572 and AUP00001626).

5.2.1. Sample collection, bacterial isolation, and identification

To isolate commensal bacteria, cecal digesta were collected from broilers and layers raised on commercial farms across Alberta. Isolation, culturing, and identification procedures of bacteria were described previously [4]. Briefly, chickens were humanely euthanized and cecal digesta were collected into liquid casein yeast media containing 30% glycerol and 0.05% cysteine (LCY), homogenized, and stored at -80ºC until cultivation. Samples were serial diluted in sterile 1 x PBS, plated on different media with various atmospheric conditions, and incubated for 72 h at 37ºC. Individual colonies were collected and the 16S ribosomal RNA gene was amplified, sequenced by Sanger method and taxonomy of sequences were assigned as described previously [4]. Genomic DNA from isolates were extracted using the Wizard® Genomic DNA purification kit. Libraries for the whole genome sequencing were constructed using NEBNext® Ultra ™ II DNA Library Prep kit (New England Biolabs Inc., CA), which were sequenced by 150 bp paired-end sequencing on an Illumina NovaSeq 6000 platform (Illumina Inc., USA). Sequence quality evaluation, trimming of adapters, assembly of draft genomes and evaluation of the quality of assembled genomes were performed using FastQC (v.0.11.9) [21], Trimmomatic (v. 039) [22], SPAdes assembler v.30.10.1 [23], and QUAST [24], respectively. Genemark [25] was used to identify

coding genes, and amino acid sequences were aligned using the BLASTp program [26] against the CAZyme database for the identification of enzymes that degrade glycosidic bonds [27].

5.2.2. DC preparation

Species to be incorporated into the DC were selected based on the collection of isolates, their ability to easily grow in the lab, their abundance within the broiler cecal microbiota, and their colonization ability according to our previous findings [4, 8]. The DC was comprised of selected isolates including *[Ruminococcus] torques*, *Alistipes finegoldii*, *Bacteroides gallinaceum*, *Bacteroides meditarraneensis*, *Bacteroides uniformis*, *P. vulgatus*, *Barnesiella viscericola, Fournierella massiliensis*, *Ligilactobacillus agilis*, *Ligilactobacillus aviarius*, *Lactobacillus crispatus*, and *Subdoligranulum variabile*. In addition, *M. hypermegale* was added to the DC community which was defined as the treatment DC+Mega. All isolates were cultured in fastidious anaerobe (FA) agar (Neogen, US), excepted for *Lactobacillus* and *Ligilactobacillus* species, which were cultured on de Man Rogosa and Sharpe (MRS) agar (BD Difco, US). Cultures were incubated under anaerobic conditions (5% CO_2, 5% H_2, and 90% N_2) in an anaerobic chamber (Bactron300, Sheldon Manufacturing, USA) for 48 h at 37ºC. Colonies were picked and re-inoculated into FA or MRS broth and agar plates. The broth or plate washed with LCY were mixed with 50% glycerol at 1:1 volume, aliquoted into 1.5 ml tubes to make glycerol stocks and stored at -80ºC. Prior to being inoculated to birds, glycerol stocks of each isolate were thawed on ice in an anaerobic chamber and an equal volume of each isolate were mixed. Fresh *M. hypermegale* cultured in FA broth was added to the DC mixture for the DC+Mega treatment.

Salmonella enterica subsp. *enterica* ser. Enteriditis SGSC 4901 (PT4) and *Salmonella enterica* subsp. *enterica* ser. Typhimurium ATCC SL1344 were streaked on fresh xylose lysine

deoxy chocolate agar (XLD) plates (Thermo Scientific) and incubated for 18 h at 37 ºC. A single colony was selected, inoculated in 5 ml FA broth, and incubated for 18 h at 37 ºC. After incubation, broth was serial diluted in sterile 1 x PBS, which was subsequently spread plated onto XLD plates and incubated for 18 h at 37 ºC for bacterial enumeration. The broth was diluted to achieve 1 x 10^8 CFUs/ml.

5.2.3. M. hypermegale survival and Salmonella inhibition

As part of the screening process to evaluate *M. hypermegale* as a potential probiotic, the tolerance of *M. hypermegale* to acids, bile and oxygen was evaluated. *M. hypermegale* was seeded into 5 ml of brain heart infusion broth (BHI, Oxoid, CA) and incubated at 37°C for 48 h. For acid tolerance assay, 500 µl of the seeded broth was inoculated into 4.5 ml of BHI broth adjusted to pH 2, 3, 5, and 7. For bile tolerance assay, 500 µl of the seeded broth was inoculated into BHI broth containing 0.3%, 0.6%, and 1.2% of porcine bile. All samples were incubated anaerobically at 37°C for 3h as determined based on the average transit time of digesta through the chicken gizzard and small intestine [28]. For oxygen tolerance assay, 500 µl of the seeded broth was inoculated into 4.5 ml of BHI broth and incubated aerobically at 37°C for 15, 30, and 60 min. A control sample not exposed to oxygen was considered as time 0. After treatments, samples were plated on BHI agar and incubated anaerobically at 37°C for 48 h.

The ability of *M. hypermegale* to inhibit *S.* Typhimurium growth was investigated using *in vitro* assays. Broth cultures containing 10^4 CFUs/ml of *S.* Typhimurium or *M. hypermegale* were co-inoculated into BHI broth. A BHI broth inoculated with 10^4 CFUs/ml of *S.* Typhimurium was used as a control. Samples were incubated anaerobically at 37°C for 48 h, then plated on XLD agar for *S.* Typhimurium enumeration. The inhibition effect was determined by comparing the *S.*

Typhimurium load in the co-culture relative to the control sample. The inhibitory effect of *M. hypermegale* on *S.* Typhimurium was also tested using the "agar slab method" [29]. Briefly, broth containing 10^4 CFUs/ml of *M. hypermegale* was spread onto BHI agar, incubated anaerobically at 37°C for 48 h, and agar slabs measuring 9 mm in diameter were cut and placed onto a BHI plate spread with *S.* Typhimurium, which was further incubated at 37°C for 24 h to measure the zone of inhibition.

5.2.4. Animal housing and study design

Day-old broilers (Ross 708, Aviagen, Huntsville, AL) obtained from a commercial hatchery were weighted, tagged with individual IDs, and randomly distributed into two-level individually ventilated isolators (GR1800 double decker Sealsafe® plus, Tecniplast, CA) lined with sterile aspen shavings. Three chicks were housed in each isolator with *ad libitum* access to water and food (Laboratory Chick Diet S-G 5065, LabDiet, MO, US) throughout the experiment. Isolators were changed as needed and 50 g of bedding materials from the previous isolator were transferred to new isolators to promote exposures to seeded microorganisms. All procedures were performed in a biosafety cabinet under specific pathogen-free conditions. Isolators were kept in a temperature-controlled room, with a daily lighting schedule of 12 h light. Room temperature was kept at 30°C for the first three days of age and then gradually reduced to 24°C as birds aged. At the beginning of each experiment, ten chicks were euthanized at arrival and cecal samples were collected and plated on XLD agar to confirm *Salmonella* absence.

In a preliminary experiment (E1), 60 day-old chicks weighing 47.24 ± 7.25 g (mean ± standard deviation (SD)) were randomly distributed into isolators (3 birds/isolator) and allocated into four treatments: Control, Mega, DC and DC+Mega. Control chicks were inoculated with

sterile LCY; chicks in the Mega treatment were inoculated with frozen *M. hypermegale* glycerol stock containing $1x10^3$ CFU/ml of cells; chicks in the DC treatment were inoculated with DC isolates, while chicks in the DC+Mega treatment were inoculated with DC isolates and *M. hypermegale* glycerol stock. All inoculations were performed the day after arrival via oral gavage with 150 µl of inocula.

Results from E1 indicated that *M. hypermegale* inoculated as a frozen glycerol stock failed to colonize the chicken gut, thus, a follow-up experiment (E2) was designed to test the colonization ability of *M. hypermegale* when provided as a fresh broth culture. Specifically, a total of 48 day-old chicks weighing 44.9 ± 3.8 g (mean ± SD) were randomly distributed into isolators and assigned to Control or Mega treatments. Chicks in the Control treatment were inoculated with sterile LCY, while chicks in the Mega treatment were inoculated with 150 µl of fresh *M. hypermegale* broth containing $1x10^3$ CFU/ml. Inoculations were performed at the day of arrival and repeated at 48 h after arrival. Two days after the repeated inoculation, chicks in all treatments were infected with *S.* Enteritidis by oral gavage with $1.5 x10^6$ cells/bird. On day 3 post infection, the lightest chick in each cage was selected for sampling, while the 2 remaining chicks were sampled 10 days post infection.

Results of E2 indicated that *M. hypermegale* successfully colonized the gut when introduced as a fresh broth culture, and a third experiment (E3) was conducted using fresh broth culture of *M. hypermegale*. A total of 72 day-old chicks weighing an average of 46.7 ± 3.7 g (mean ± SD) were randomly distributed into twenty-four cages (3 birds per cage) and assigned to three treatments: Control, DC and DC+Mega (8 cages per treatment). Control chicks were inoculated with sterile LCY; chicks in the DC treatment were inoculated with DC isolates; and chicks in the DC+Mega treatment were inoculated with DC isolates and fresh *M. hypermegale* broth ($1x10^3$

CFU/ml). Two days after the repeated inoculation, chicks in all treatments were infected by oral gavage with 150 µl of $1x10^7$ CFU/ml *S.* Enteritidis PT4. On day 3 post-infection, the lightest chick in each cage was selected for sampling, while the 2 remaining chicks were sampled 10 days post-infection.

5.2.5. Sampling

Chicks were euthanized by cervical dislocation, and the coelomic cavity was opened using sterile technique. The whole spleen was collected into 1 x PBS and stored on ice for *Salmonella* enumeration. Blood samples were collected by cardiac puncture and stored on ice for whole blood bactericidal assay as a proxy for innate immunity [30]. Whole blood samples were diluted to 1:4 and 1:20 with pre-warmed (40ºC) CO_2-independent media containing 4mM L-glutamine. Diluted blood samples (90µl) were mixed with 10 µl *S.* Enteritidis broth culture containing 10 CFU/µl. The mixtures were immediately plated (Time 0) or incubated at 37ºC for 30 min (Time 30) and then plated on XLD. *Salmonella* enumeration on XLD plates was performed after 24 h incubation at 37 ºC.

Approximately 100 mg of cecal digesta were collected and immediately stored at -80°C for DNA extraction or collected into LCY and stored on ice for bacterial culturing and *Salmonella* enumeration. Concentrations of short-chain fatty acids in cecal digesta were determined by gas chromatography as described previously [8]. Levels of cytokine/chemokine in cecal tissue were determined by a multiplex cytokine assay (Featured – Chicken Cytokine/ Chemokine 12-Plex Assay, Eve Technologies Corporation, CA) as described previously [8].

5.2.6. Salmonella and Megamonas quantification

Spleen and digesta samples collected in 1 x PBS were weighed, homogenized by shaking twice in a tridimensional motion at 6 m/sec for 40 s using a bead-beater (FastPrep-24TM 5G, MP Biomedicals), serial diluted and plated on XLD agar plates, which were incubated for 48 h at 37ºC for *Salmonella* enumeration. Quantitative RT-PCR was used to determine the abundance of *Salmonella* and *Megamonas* in cecal contents. To generate a standard curve, DNA was extracted from pure broth cultures of *S.* Enteritidis and *M. hypermegale* using a Wizard Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's protocol. The *Salmonella* enterotoxin gene (*stn*) gene was amplified using primers: forward primer, 5'-CTTTGGTCGTAAAATAAGGCG-3' and reverse primer, 5'-TGCCCAAAGCAGAGAGATTC-3'. The quantification of *Megamonas* was performed using primers: forward primer, 5'-GGGTGCTAATACCGAATGAAT-3' and reverse primer, 5'-CGTGTCTACGTCCCAATGTG-3'. PCR reaction mixtures contained 5 µl of SYBR Green SuperMix (Quantabio, US), 0.5 µl of each forward and reverse primer, 3 µl of nuclease free water, and 1 µl of DNA diluted to a concentration of 5 ng/µl. The PCR programs consisted of an initial denaturation step of 3 min at 95ºC, followed by 40 cycles of 95ºC for 10 s and 60ºC for 30 s, which was performed on an ABI StepOne™ real-time System (Applied Biosystems, Foster City, CA).

5.2.6. DNA extraction and 16S rRNA gene amplicon sequencing analysis

Total DNA from cecal digesta samples was extracted using a QIAamp DNA stool mini kit (Qiagen NV, Netherlands), following the manufacturers' Pathogen Detection protocol, with minor modifications. Specifically, approximately 100 mg of digesta content was mixed with the Inhibitex® buffer and 2.0 mm garnet beads (BioSpec Products, Bartlesville, OK), homogenized

and lysed by bead-beating twice at 6.0 m/s for 30 s. DNA concentrations were determined using a Quant-iT™ Picogreen™ dsDNA assay kit (Invitrogen, Thermo Fisher Scientific, US).

Amplicon libraries targeting the V3-V4 regions of the *16S rRNA* gene were prepared following the Illumina 16S Metagenomic Sequencing Library Preparation protocol (#15044223 Rev.B). Sequencing was performed on an Illumina Miseq platform (Illumina Inc, San Diego, CA) using 2x 300 cycles. Raw sequences were processed using Quantitative Insights into Microbial Ecology 2 v2020.2 (QIIME2, [30]). Forward and reverse sequences were denoised and truncated at 270 and 220 bp, respectively; and chimeras were removed using DADA2 (v. 2020.2.0) plugin [31]. Multiple sequence alignments were performed using MAFFT [32] and phylogenetic trees were generated using FastTree method [33]. Naïve Bayes classifier [34] pretrained on SILVA 138 QIIME compatible database [35] was used for taxonomic classification, and sequences were clustered at 99% identity using majority taxonomy strings. Downstream analyses were performed using phyloseq v.1.40.0 [36], microbiome v.1.18.0 [37] and qiime2R v.0.99.6 [38, 39] packages in R v 1.4.1717 [39]. Amplicon sequence variants (ASVs) assigned to Mitochondria family, Chloroplast order, Archaea kingdom, Unassigned at the phylum level and present in less than 10% of the samples or presenting less than 10 reads were removed from the dataset. Reads were rarefied at an even count before downstream analysis. Alpha-diversity was evaluated using phylogenetic diversity (PD) and Chao1 indexes. Beta-diversity was evaluated using Bray-Curtis distance matrix and visualized by principal coordinates analysis (PCoA). Differentially abundant taxa were identified using DESeq2 with apeglm for logarithmic fold change shrinkage and FDR correction analysis [40, 41]. Analysis at the taxa level was performed by merging all the ASVs exhibiting the same taxonomy string using *tax_glom* function (phyloseq package). Spearman correlation analysis

was performed using psych v.2.3.3 package [42]. Figures were generated using ggplot2 v.3.4.0 [43].

5.2.7. Colonization ability and efficiency

Colonization ability of bacterial isolates was determined based on the presence/absence of species in the cecal samples of inoculated birds. Specifically, species that were detected in at least half of the inoculated birds were considered as good colonizers, whereas species detected in less than half of the birds were considered as poor colonizers. Colonization efficiency was subsequently determined by dividing the average number of reads of each species in the inoculated birds by the number of reads of that species in the inocula. Colonization efficiency results lower than 0.5, between 0.5 and 1, and higher than 1 were classified as low, medium, and high efficiency colonizers, respectively.

5.2.8. Statistical analyses

Statistical analyses of beta-diversity matrices were performed using multivariate homogeneity of group dispersions and permutational multivariate analysis of variance with Benjamin-Hochberg procedure for FDR control. Data were tested for normality using the Shapiro-Wilk test and analyzed by one-way ANOVA followed by Tukey's HSD test if normally distributed, or Kruskal-Wallis and pairwise Wilcoxon test with FDR adjustment for multiple comparisons if distribution was abnormal. A *p*-value of less than 0.05 was considered as statistically significant.

5.3. RESULTS

There were no differences in the body weight among treatments in any of the timepoints measured in all trials (Table 5.1).

5.3.1. Salmonella growth is inhibited by co-culturing with M. hypermegale in vitro

M. hypermegale can endure pH 5, at least 30-min of oxygen exposure, and up to 1.2% bile acid in the media. There was no difference on viable cells between pH 5 and 7 ($p = 0.943$), while no survival was observed at pH 2 or 3. The presence of up to 1.2% of bile in the media had no effects on *M. hypermegale* survival ($p = 0.374$). Oxygen exposure for 60 min reduced the number of viable cells of *M. hypermegale* ($p = 0.047$) by 41.7%, but no effects on survival were observed after 15- and 30-min of oxygen exposure compared to starting bacterial abundance (time 0). Co-culture of *M. hypermegale* and *Salmonella* reduced *Salmonella* counts by 99.3% ($p = 0.003$); however, no inhibition zone was observed using the agar slab method.

5.3.2. The DC increased the PD in the ceca, and M. hypermegale was shown to be a good and efficient colonizer, but only when introduced as fresh culture

In E1, *M. hypermegale* introduced from a frozen glycerol stock failed to colonize the chicken ceca and was not detected in the Mega or DC+Mega treated birds. At day 14, beta-diversity analysis indicated that the cecal microbiota of Control and Mega birds was significantly different from that of DC and DC+Mega birds ($r^2=0.70$, $p = 0.001$); however, no differences were found in the cecal microbiota between Control and Mega birds, nor between DC and DC+Mega birds (Figure 5.1A). The DC+Mega birds presented higher PD than that of Control ($p = 0.03$) and Mega ($p = 0.01$) birds, and no differences were observed in Chao1 index ($p = 0.026$) (Figure 5.2A). In

E2, *M. hypermegale* was introduced from a fresh culture successfully colonized the chicken gut. Community composition, as indicated by beta-diversity matrix was significantly different between Control and Mega birds ($r^2 = 0.60$, $p = 0.001$) (Figure 5.1B). No differences in PD and Chao1 index ($p = 0.355$, $p = 0.96$, respectively) were found between Control and Mega birds (Figures 5.2B). In E3, birds treated with DC or DC+Mega presented higher PD than the Control group ($p < 0.001$). Chao1 index was higher in DC+Mega compared to Control group ($p < 0.001$) (Figure 5.2C). Beta-diversity analysis indicated that Control group was different from DC and DC+Mega (r2 = 0.64, $p = 0.001$ and $r^2 = 0.71$ and $p = 0.001$, respectively), and that DC and DC+Mega communities were also significantly different ($r^2 = 0.26$, $p = 0.001$) (Figure 5.1C).

In E1 and E3, an average of 94% of the cecal community of DC and DC+Mega groups were bacteria found in the DC inoculum, while only 15% of the cecal community in Control birds was shared with the DC inoculum including three species: *F. massiliensis* detected in one bird in E1; *L. agilis* detected in five birds in E3, and *L. crispatus* consistently detected in all Control birds from E3. Therefore, *L. crispatus* was considered to be part of the baseline/initial microbiota in E3 (Figure 5.3). The cecal microbiota of Control birds was dominated by Firmicutes (an average of 51%) and Proteobacteria (an average of 49%); whereas the cecal microbiota of DC and DC+Mega birds were dominated by Bacteroidetes (an average of 86% and 76%, respectively), Firmicutes (an average of 9% and 25%, respectively) and Proteobacteria (an average of 5% and 2%, respectively).

In E2, *M. hypermegale* given as a fresh culture successfully colonized the ceca of Mega-treated birds and its relative abundance ranged from 18% to 73%, with an average of 57% (Figure 5.4A). *M. hypermegale*-treated birds presented less *Enterococcus* ($p = 0.001$) and *Escherichia-Shigella* than Control birds ($p < 0.001$). There were no differences in cecal *Salmonella* load as

determined by qPCR (Figure 5.4B); however, the enumeration technique indicated that *Salmonella* load was higher in the spleen of Mega-treated birds ($p = 0.002$, Figure 5.4C).

In E3, differential abundance analysis indicated that DC and DC+Mega birds presented more *B. gallinaceum*, *B. uniformis*, *A. finegoldii*, *P. vulgatus*, and *B. viscericola*, but less *Escherichia-Shigella*, *Proteus*, *Enterococcus*, *Clostridium sensu stricto 1*, and *L. crispatus* than birds in the Control group. In addition, DC+Mega birds presented lower relative abundance of *Salmonella*, and higher levels of *F. massiliensis*, *R. torques*, and *M. hypermegale* than Control birds. Comparisons between DC and DC+Mega treatments indicated DC cecal microbiota to be enriched for *Escherichia-Shigella* and *L. agilis*, whereas the cecal microbiota of DC+Mega showed enriched *M. hypermegale*, *Clostridium sensu stricto 1*, *R. torques*, and *F. massiliensis* (Figure 5.5).

DESeq2 results indicated lower relative abundance of *Salmonella* in the DC+Mega treatment. While the mean Salmonella loads by qPCR were in the same direction as the 16S amplicon sequencing data, no differences in cecal *Salmonella* load were found ($p = 0.76$) (Figure 5.6A). Salmonella load in the spleen was not different ($p = 0.08$) with most samples being undetected for *Salmonella* (Figure 5.6B). Correlation analysis performed between taxa present in the inocula and the baseline microbiota indicated *M. hypermegale* to be negatively correlated with *Escherichia-Shigella*, whereas *Salmonella* was negatively associated with *B. viscericola* and positively associated with *P. vulgatus* and *A. finegoldii*.

Birds in the DC+Mega treatment showed higher IFN-γ ($p = 0.028$) in cecal tissues than birds in the Control group. Cecal tissue from birds in the DC treatment exhibited the lowest M-CSF concentration among treatment groups ($p = 0.005$) (Figure 5.6C), which may coincide with the lower relative abundance of *Salmonella* and the pattern of lower spleen translocation in this group. Cecal concentration of isovalerate was higher in birds from DC and DC+Mega treatments

compared with Control. Valerate concentration was higher in DC+Mega compared to Control birds ($p = 0.045$), and propionate ($p < 0.001$) concentration was higher in DC+Mega treated birds compared to birds in Control and DC treatments (Figure 5.7).

5.3.3. B. viscericola and P. vulgatus are good colonizers of the chicken ceca

Colonization ability and efficiency of bacteria in the inocula were determined based on the presence of isolates in the ceca of inoculated birds as well as on the ratio between the number of reads in samples and in the inocula. *A. finegoldii*, *B. gallinaceum*, *B. viscericola*, *P. vulgatus*, *L. crispatus*, and *L. agilis* were detected in more than half of the cecal samples of inoculated birds and deemed to be good colonizers. Within the good colonizers group, *B. viscericola*, *P. vulgatus*, *L. crispatus*, and *L. agilis* were detected in all birds. The ratio between the average number of reads in samples and in inocula was higher than 1 for *B. viscericola* and *P. vulgatus* which were considered as highly efficient colonizers, whereas the efficiency of colonization for *A. finegoldii*, *L. crispatus*, and *L. agilis* were deemed as low. Although *B. uniformis* failed to colonize more than half of the inoculated birds, once introduced, the observed reads in inoculated birds were higher than that in the inocula, thus this isolate was considered as a poor colonizer, but with high colonization efficiency. *B. mediterraneesis*, *L. aviarius*, and *S. variabile* failed to colonize the chicken gut and were not detected in the ceca of inoculated birds. There were inconsistencies between observed colonization abilities and efficiencies for *[Ruminococcus] torques* and *Fournierella massiliensis* amongst studies. *M. hypermegale* failed to colonize when introduced from a frozen glycerol stock in E1 but colonized all the birds and was a good and highly efficient colonizer when introduced as fresh culture in E2 and E3. *B. mediterraneensis*, *L. aviarius*, and *S. variabile* consistently failed to colonize the chicken gut (Figure 5.8).

5.4. DISCUSSION

In this study, a DC containing 12 bacterial isolates harvested from adult chicken ceca was inoculated to young chicks, resulting in increased alpha-diversity and substantial changes in the cecal microbiota composition evaluated at 14 days old in the context of *Salmonella* challenge. Strikingly, more than 75% of the cecal microbiota of DC-inoculated birds was composed by Bacteroidetes, which were completely absent in Control birds. Bacteroidetes species were reported to be host-adapted [44] and *Alistipes*, *Bacteroides*, *Barnesiella*, and *Phocaeicola* were found to be good colonizers of the chicken ceca, either when introduced as isolates or as part of complex and defined communities [10, 44–46]. Bacteroidetes members are non-spore forming and sensitive to oxygen, therefore, they have low ability to survive in the environment and are likely to be lost, reduced, or colonized with delay in broilers due to biosafety practices employed in the poultry industry, which hinder the contact between newly hatched chicks and their parental microbiota [1, 47]. Consequently, it is possible that once Bacteroidetes are introduced to the gut environment, they can occupy available niches and efficiently engraft. Despite the substantial difference in cecal microbiota composition, the differences in host responses measured in inoculated and Control birds were subtle. Inoculated birds presented higher concentrations of IFN-γ, M-CSF, propionic acid, isovaleric and valeric acid in ceca with no significant differences in body weight, *Salmonella* load, other cytokines measured, and whole blood bactericidal capacity. Similarly, a previous study found that the inoculation of nine bacteria obtained from chickens affected the chick gut microbiota but caused only a transient increase in systemic IgA levels [48].

We speculated two main reasons why the inoculation with DC did not significantly impact *Salmonella* load compared with Control birds. First, the DC we inoculated had a limited number

of microorganisms. Significant reductions in *Salmonella* load were usually observed in studies inoculating defined communities containing 25 species or more [18, 49, 50], or using commercial products [51] from which the bacterial composition is not disclosed but is likely to include diverse species. As an exception, a study found that inoculation with ten bacterial isolates harvested from the ceca of feral chickens resulted in a significant reduction in *Salmonella* load and ameliorated intestinal inflammation [52]. In that way, rather than a low number of microorganisms, the microorganisms selected to include in our DC may not be effective to promote *Salmonella* resistance. Our second speculation relies on the fact that the baseline microbiota of our birds was enriched with *Escherichia-Shigella* which made up an average of 42.0 ± 12.0 % of the cecal microbial community in Control birds; however, in birds inoculated with DC and DC+Mega, the average relative abundance of *Escherichia-Shigella* was less than 5%. It is possible to speculate that the high abundance of *Escherichia-Shigella* within the ceca of Control birds had promoted a similar level of protection of hosts to *Salmonella* infection to that of the DC or DC+Mega treatment. Commensal *Enterobacteriaceae* were previously demonstrated to protect chickens against *Salmonella* infection through oxygen competition [53]. The proposed mechanism indicated that, in the presence of inflammatory signalling, butyrate produced by Clostridia stimulate Peroxisome Proliferatior-Activated Receptor gamma signalling pathway that results in mitochondrial oxidation and epithelial hypoxia [53]. The maintenance of epithelial hypoxia by Clostridia and the consumption of oxygen by *Enterobacteriaceae* hindered the ability of *Salmonella* to perform aerobic respiration with decreased colonization ability [53]. Interestingly, *Clostridium Stricto Sensu 1* made up an average of 16.1% of the microbial community in the Control birds, but with a significantly lower relative abundance in DC and DC+Mega groups (0.8% and 2.3%, respectively. Further experiments would be necessary to confirm this speculation. The

finding that DC and DC+Mega reduced the abundance of *Escherichia-Shigella* could be considered a potentially positive outcome. Currently, avian pathogenic *Escherichia coli* is a leading cause of antibiotic treatment in broiler flocks in Alberta [54], and the use of DC could be tested as a strategy to mitigate the occurrence of *Escherichia-Shigella-associated* diseases.

M. hypermegale provided from a frozen glycerol stock failed to colonize birds. This was largely unexpected, as *Megamonas* has been shown to be a good colonizer in birds inoculated with frozen cecal contents, cecal cultures, and competitive exclusion products [8, 55]. Even if accidentally exposed to oxygen during the inoculation procedure, the *M. hypermegale* strain used in our study was shown to survive for at least 30 minutes. Therefore, it is more likely that the *M. hypermegale* glycerol stock was affected by the freeze-thaw process, since fresh *M. hypermegale* provided at a low dose successfully colonized the birds. Freeze-thaw process could also have affected the other three species that failed to colonize (*B. mediterraneensis*, *S. variabile* and *L. aviarius*), and this will be elucidated by providing fresh cultures of these isolates in the future.

In the E2, birds in the Mega treatment had higher *Salmonella* counts in the spleen than Control birds, indicating that the *M. hypermegale* strain might not be beneficial to birds in the context of not being introduced as part of a microbial community. Birds colonized with DC+Mega had increased valerate, isovaleric, and propionate, which might be related to the presence of *M. hypermegale* as it is a propionate producer and metabolizes free H_2 favouring the production of SCFAs by other members of the community [17, 56]. In a previous experiment, we found that the concentrations of valerate and propionate were higher in cecal contents of chicks inoculated with microbial cultures and cecal contents from adult birds [8], which agrees with the present study. This suggests that inoculation with the DC community partially recapitulated the effects of introducing a complex microbial community regarding the production of short-chain fatty acids.

The *in vitro* assays of *M. hypermegale* showed some contradictory results. The acid tolerance assay indicated that pH 2 or 3 killed *M. hypermegale*, which is somewhat contradictory to the observed *M. hypermegale*'s survival rate *in vivo*. This difference might be due to the 3-h incubation period used in the *in vitro* assay being longer than the retention time within the gizzard and proventriculus *in vivo*. Nine-day-old chickens have retention times ranging from 55 to 480 minutes [57], and therefore, future *in vitro* acid tolerance assays should be optimized to mimic *in vivo* conditions accordingly. In addition, the presence of digesta can promote increased acid tolerance by buffering pH and providing nutrients, such as glucose, which can improve bacterial survival [58]. Nonetheless, given *M. hypermegale* provided as a fresh broth culture colonized all inoculated birds at relatively high rates, it is safe to state that it presents sufficient resistance to acidic conditions. Another conflicting result was observed in *in vitro* inhibition of *Salmonella* by *M. hypermegale*. Co-culture assay indicated inhibitory effect of *Salmonella* by *M. hypermegale*, which was not observed using the slab method. These phenotypes might be explained by differences in metabolism of bacteria when growing in solid or liquid media [59].

5.5. CONCLUSION

In conclusion, we found that the introduction of a DC containing 12 bacterial species caused substantial changes in cecal microbiota composition without causing large effects on host physiology and ability to resist *Salmonella* challenge. We have identified *A. finegoldii*, *B. gallinaceum*, *B. viscericola*, *P. vulgatus*, *L. crispatus*, and *L. agilis* as good colonizers of the chicken gut and found that the introduction of bacteria harvest from adult chicken ceca causes significant reduction in the relative abundance of *Escherichia-Shigella*.

5.6. REFERENCES

1. Nurmi E, Rantala M. New aspects of salmonella infection in broiler production. Nature. 1973;241:210–1.

2. Rantala M, Nurmi E. Prevention of the growth of salmonella infantis in chicks by the flora of the alimentary tract of chickens. British Poultry Science. 1973;14:627–30.

3. Hird SM. Evolutionary Biology Needs Wild Microbiomes. Front Microbiol. 2017;8.

4. Marcolla C, Ju T, Lantz HL, Willing BP. Investigating the cecal microbiota of broilers raised in extensive and intensive production systems. Res Sq. 2023.

5. Seidlerova Z, Kubasova T, Faldynova M, Crhanova M, Karasova D, Babak V, et al. Environmental impact on differential composition of gut microbiota in indoor chickens in commercial production and outdoor, backyard chickens. Microorganisms. 2020;8.

6. Ramírez GA, Richardson E, Clark J, Keshri J, Drechsler Y, Berrang ME, et al. Broiler chickens and early life programming: Microbiome transplant-induced cecal community dynamics and phenotypic effects. PLoS One. 2020;15 11 November.

7. Ocejo M, Oporto B, Hurtado A. 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. Sci Rep. 2019;9.

8. Marcolla CS, Ju T, Willing BP. Cecal Microbiota Development and Physiological Responses of Broilers Following Early Life Microbial Inoculation Using Different Delivery Methods and Microbial Sources. Appl Environ Microbiol. 2023;89.

9. Medvecky M, Cejkova D, Polansky O, Karasova D, Kubasova T, Cizek A, et al. Whole genome sequencing and function prediction of 133 gut anaerobes isolated from chicken caecum in pure cultures. BMC Genomics. 2018;19.

10. Poudel B, Shterzer N, Sbehat Y, Ben-Porat N, Rakover M, Tovy-Sharon R, et al. Characterizing the chicken gut colonization ability of a diverse group of bacteria. Poult Sci. 2022;101.

11. Sakon H, Nagai F, Morotomi M, Tanaka R. Sutterella parvirubra sp. nov. and Megamonas funiformis sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2008;58:970–5.

12. Yadav S, Caliboso KD, Nanquil JE, Zhang J, Kae H, Neupane K, et al. Cecal microbiome profile of Hawaiian feral chickens and pasture-raised broiler (commercial) chickens determined using 16S rRNA amplicon sequencing. Poult Sci. 2021;100.

13. Scupham AJ, Patton TG, Bent E, Bayles DO. Comparison of the cecal microbiota of domestic and wild turkeys. Microb Ecol. 2008;56:322–31.

14. Wienemann T, Schmitt-Wagner D, Meuser K, Segelbacher G, Schink B, Brune A, et al. The bacterial microbiota in the ceca of Capercaillie (*Tetrao urogallus*) differs between wild and captive birds. Syst Appl Microbiol. 2011;34:542–51.

15. Harrison AP, Hansen PA. Bacteroides hypermegas nov. spec. Antonie Van Leeuwenhoek. 1963;29:22–8.

16. Ella Barnes BM, Impey CS, H Stevens A BJ. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. 1979.

17. Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW, Pallen MJ. Extensive microbial and functional diversity within the chicken cecal microbiome. PLoS One. 2014;9.

18. Impey CS, Mead GC, George SM. Competitive exclusion of salmonellas from the chick caecum using a defined mixture of bacterial isolates from the caecal microflora of an adult bird. Journal of Hygiene. 1982;89:479–90.

19. Scupham AJ, Jones JA, Rettedal EA, Weber TE. Antibiotic Manipulation of Intestinal Microbiota To Identify Microbes Associated with *Campylobacter jejuni* Exclusion in Poultry. Appl Environ Microbiol. 2010;76:8026–32.

20. Zaytsoff SJM, Montina T, Boras VF, Brassard J, Moote PE, Uwiera RRE, et al. Microbiota Transplantation in Day-Old Broiler Chickens Ameliorates Necrotic Enteritis via Modulation of the Intestinal Microbiota and Host Immune Responses. Pathogens. 2022;11:972.

21. Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data . 2010.

22. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.

23. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology. 2012;19:455–77.

24. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: Quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5.

25. Besemer J, Borodovsky M. GeneMark: Web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res. 2005;33 SUPPL. 2.

26. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic Local Alignment Search Tool. J Mol Biol. 1990;215:403–10.

27. Drula E, Garron M-L, Dogan S, Lombard V, Henrissat B, Terrapon N. The carbohydrate-active enzyme database: functions and literature. Nucleic Acids Res. 2022;50:D571–7.

28. Svihus B, Itani K. Intestinal Passage and Its Relation to Digestive Processes. Journal of Applied Poultry Research. 2019;28:546–55.

29. Dec M, Puchalski A, Urban-Chmiel R, Wernicki A. Screening of Lactobacillus strains of domestic goose origin against bacterial poultry pathogens for use as probiotics. Poult Sci. 2014;93:2464–72.

30. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.

31. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.

32. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol Biol Evol. 2013;30:772–80.

33. Price MN, Dehal PS, Arkin AP. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5.

34. Pedregosa FABIANPEDREGOSA F, Michel V, Grisel OLIVIERGRISEL O, Blondel M, Prettenhofer P, Weiss R, et al. Scikit-learn: Machine Learning in Python Gaël Varoquaux Bertrand Thirion Vincent Dubourg Alexandre Passos PEDREGOSA, VAROQUAUX, GRAMFORT ET AL. Matthieu Perrot. 2011.

35. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 2013;41.

36. McMurdie PJ, Holmes S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One. 2013;8.

37. Lahti L, Shetty S. Introduction to the microbiome R package.

38. Bisanz JE. qiime2R: Importing QIIME2 artifacts and associated data into R sessions.

39. RStudio Team. RStudio: Integrated Development for R. 2020.

40. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15.

41. Zhu A, Ibrahim JG, Love MI. Heavy-Tailed prior distributions for sequence count data: Removing the noise and preserving large differences. Bioinformatics. 2019;35:2084–92.

42. Revelle W. psych: Procedures for Psychological, Psychometric, and Personality Research. 2023.

43. Wickham H. ggplot2 Elegant Graphics for Data Analysis Second Edition. 2016.

44. Kollarcikova M, Faldynova M, Matiasovicova J, Jahodarova E, Kubasova T, Seidlerova Z, et al. Different bacteroides species colonise human and chicken intestinal tract. Microorganisms. 2020;8:1–14.

45. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One. 2019;14.

46. Glendinning L, Chintoan-Uta C, Stevens MP, Watson M. Effect of cecal microbiota transplantation between different broiler breeds on the chick flora in the first week of life. Poult Sci. 2022;101:101624.

47. Karasova D, Faldynova M, Matiasovicova J, Sebkova A, Crhanova M, Kubasova T, et al. Host Species Adaptation of Obligate Gut Anaerobes Is Dependent on Their Environmental Survival. Microorganisms. 2022;10.

48. Zenner C, Hitch TCA, Riedel T, Wortmann E, Tiede S, Buhl EM, et al. Early-Life Immune System Maturation in Chickens Using a Synthetic Community of Cultured Gut Bacteria. mSystems. 2021;6.

49. Stavric S, Gleeson TM, Blanchfield B, Pivnick H. Competitive Exclusion of Salmonella from Newly Hatched Chicks by Mixtures of Pure Bacterial Cultures Isolated from Fecal and Cecal Contents of Adult Birds. J Food Prot. 1985;48:778–83.

50. Gleeson TM, Stavric S, Blanchfield B. Protection of Chicks against Salmonella Infection with a Mixture of Pure Cultures of Intestinal Bacteria. Avian Dis. 1989;33:636.

51. Kerr AK, Farrar AM, Waddell LA, Wilkins W, Wilhelm BJ, Bucher O, et al. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on Salmonella spp. prevalence and concentration in broiler chickens. Prev Vet Med. 2013;111:112–25.

52. Wongkuna S, Kumar R, Ghimire S, Mattiello Drescher S, Maji A, Ambat A, et al. Identification of a microbial sub-community from the feral chicken gut that reduces 1 Salmonella colonization and improves gut health in a gnotobiotic chicken model. 2. https://doi.org/10.1101/2022.07.23.501241.

53. Litvak Y, Mon KKZ, Nguyen H, Chanthavixay G, Liou M, Velazquez EM, et al. Commensal Enterobacteriaceae Protect against Salmonella Colonization through Oxygen Competition. Cell Host Microbe. 2019;25:128-139.e5.

54. Canadian Integrated Program for Antimicrobial Resistance Surveillance.

55. Dame-Korevaar A, Kers JG, van der Goot J, Velkers FC, Ceccarelli D, Mevius DJ, et al. Competitive Exclusion Prevents Colonization and Compartmentalization Reduces Transmission of ESBL-Producing Escherichia coli in Broilers. Front Microbiol. 2020;11.

56. Rychlik I. Composition and function of chicken gut microbiota. Animals. 2020;10.

57. Rougière N, Carré B. Comparison of gastrointestinal transit times between chickens from D + and D- genetic lines selected for divergent digestion efficiency. Animal. 2010;4:1861–72.

58. Corcoran BM, Stanton C, Fitzgerald GF, Ross RP. Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. Appl Environ Microbiol. 2005;71:3060–7.

59. Michael, Waturangi DE. Antibiofilm activity from endophyte bacteria, Vibrio cholerae strains, and actinomycetes isolates in liquid and solid culture. BMC Microbiol. 2023;23:83.

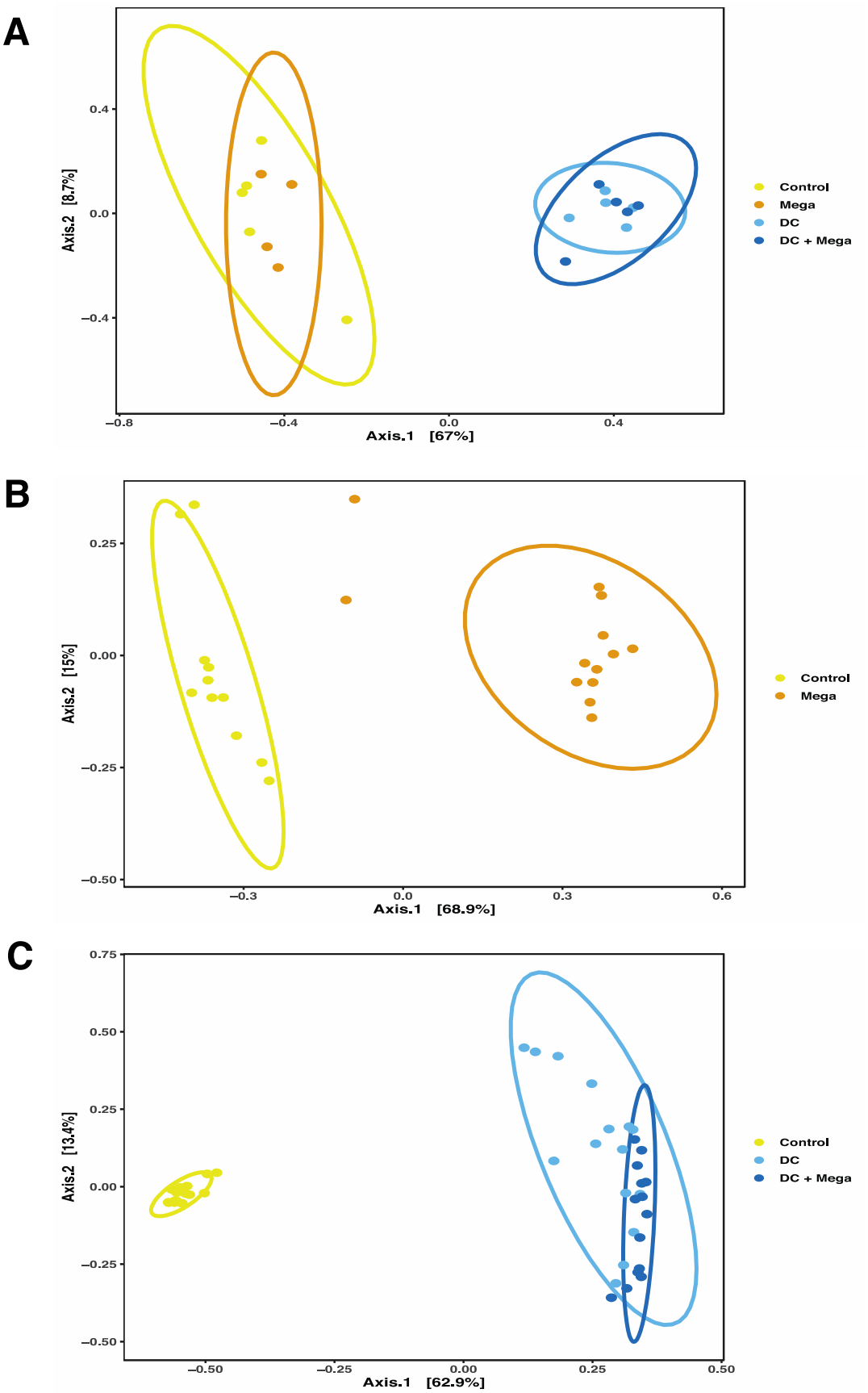

A
0.4
0.0
−0.4
Axis.2 [8.7%]
−0.8
−0.4
0.0
0.4
Axis.1 [67%]
Control
Mega
DC
DC + Mega
B
0.25
0.00
−0.25
−0.50
Axis.2 [15%]
−0.3
0.0
0.3
0.6
Axis.1 [68.9%]
Control
Mega
C
0.75
0.50
0.25
0.00
−0.25
−0.50
Axis.2 [13.4%]
−0.50
−0.25
0.00
0.25
0.50
Axis.1 [62.9%]
Control
DC
DC + Mega

Figure 5.1. Principal coordinates analysis (PCoA) generated based on Bray-Curtis dissimilarity of cecal samples obtained from 14-day-old Control chicks and chicks colonized with Mega, DC or DC+Mega treatments in E1 (A), E2 (B) and E3 (C). Samples are colored and shaped according to treatments and data ellipses represent the 95% confidence region for group clusters assuming a multivariate t-distribution.

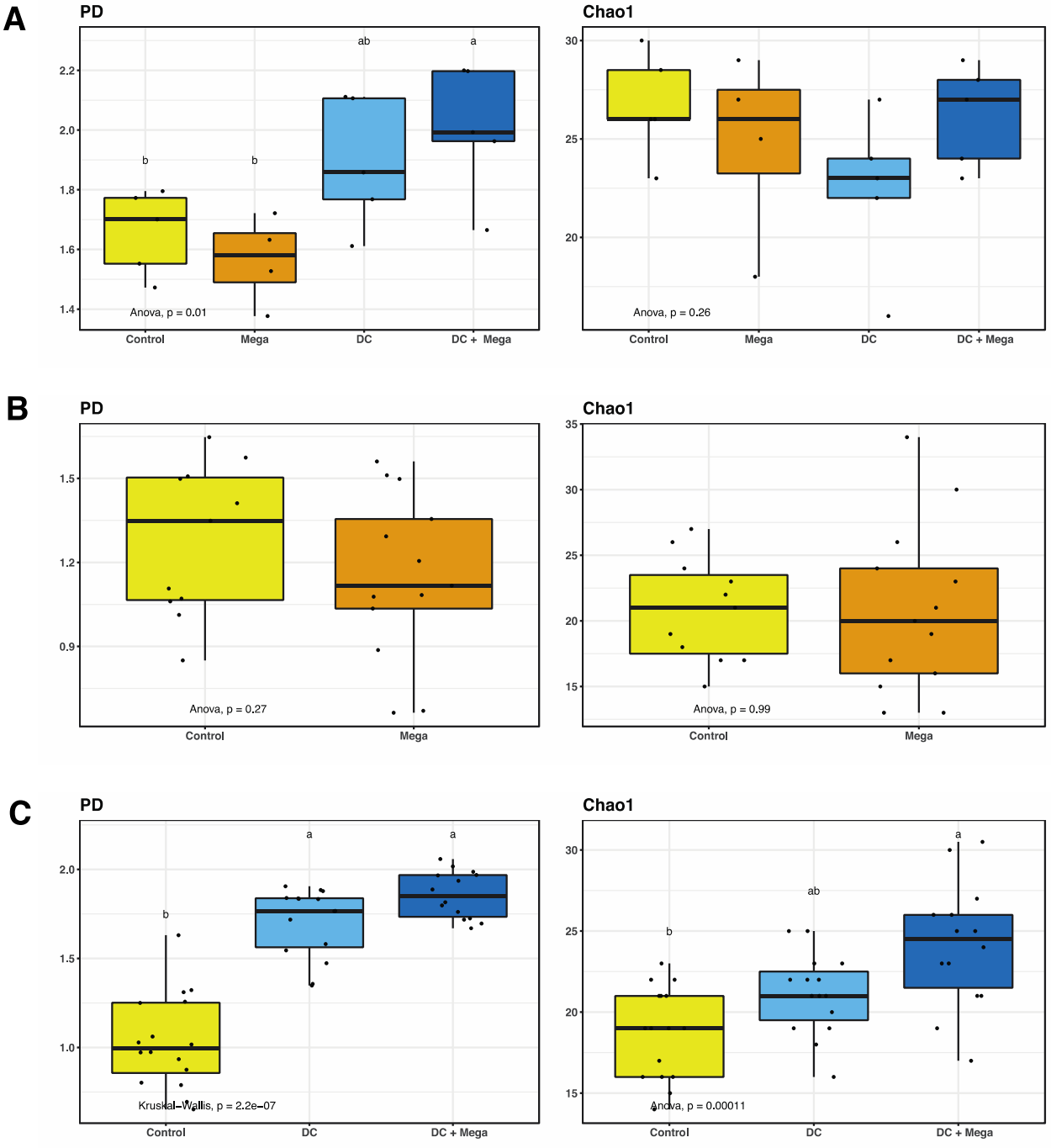


Figure 5.2. Alpha-diversity indices PD and Chao1 of cecal samples obtained from 14-day-old Control chicks and chicks colonized with Mega, DC or DC+Mega treatments in E1 (A), E2 (B) and E3 (C). [a-b] Means with no common superscript are significantly different after pairwise comparison using Tukey HSD test or Wilcoxon rank sum test with FDR correction. $\alpha = 0.05$

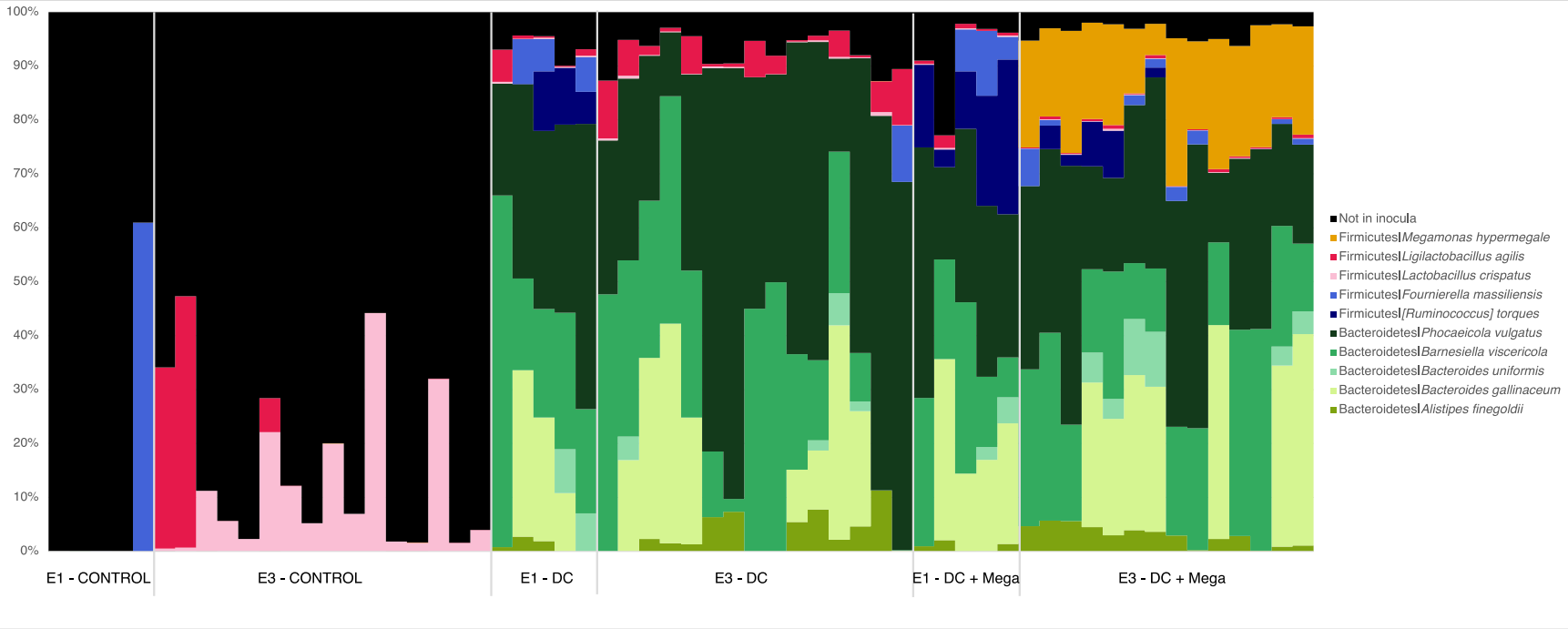


Figure 5.3. Bar plots of the relative abundances of bacterial species included in the DC and DC + Mega inocula detected in cecal samples obtained from 14-day-old chicks from Control, DC and DC + Mega treatments in E1 and E3. Species not included in the inocula were considered as baseline microbiota and combined into as "Not in inocula" and shown in black.

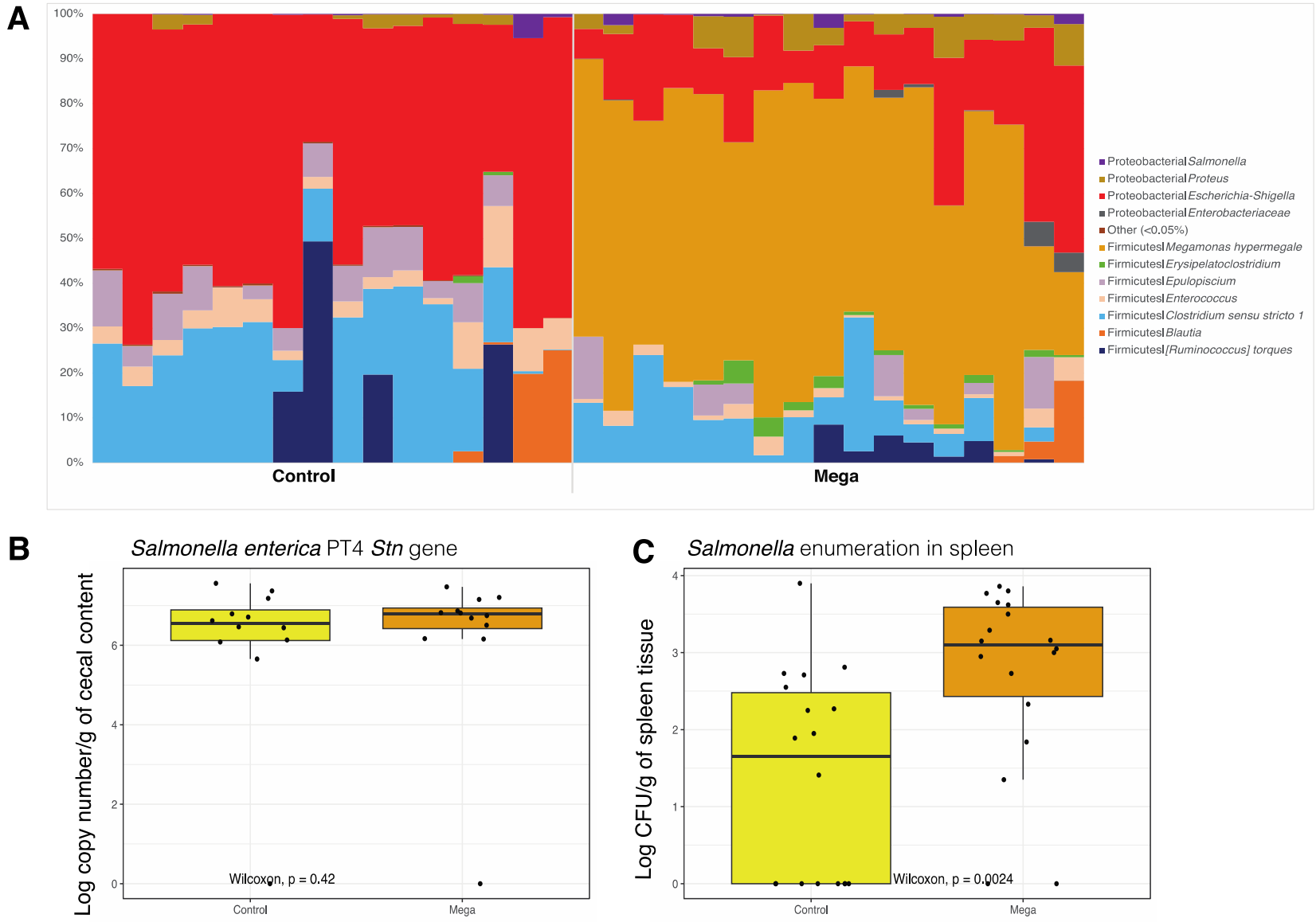

A
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Control
Mega
Proteobacterial Salmonella
Proteobacterial Proteus
Proteobacterial Escherichia-Shigella
Proteobacterial Enterobacteriaceae
Other (<0.05%)
Firmicutes| Megamonas hypermegale
Firmicutes| Erysipelatoclostridium
Firmicutes| Epulopiscium
Firmicutes| Enterococcus
Firmicutes| Clostridium sensu stricto 1
Firmicutes| Blautia
Firmicutes| [Ruminococcus] torques
B
Salmonella enterica PT4 Stn gene
Log copy number/g of cecal content
Wilcoxon, p = 0.42
Control
Mega
C
Salmonella enumeration in spleen
Log CFU/g of spleen tissue
Wilcoxon, p = 0.0024
Control
Mega

Figure 5.4. (A) Bar plots showing the relative abundances of bacterial species detected in cecal samples obtained from 14-day-old chicks from Control and Mega treatments in E2. Dendrograms showing the effect of treatments on *Salmonella* colonization in **(B)** cecal contents based on *Stn* gene quantification by qPCR and in **(C)** spleen tissues based on culturing and enumeration method.

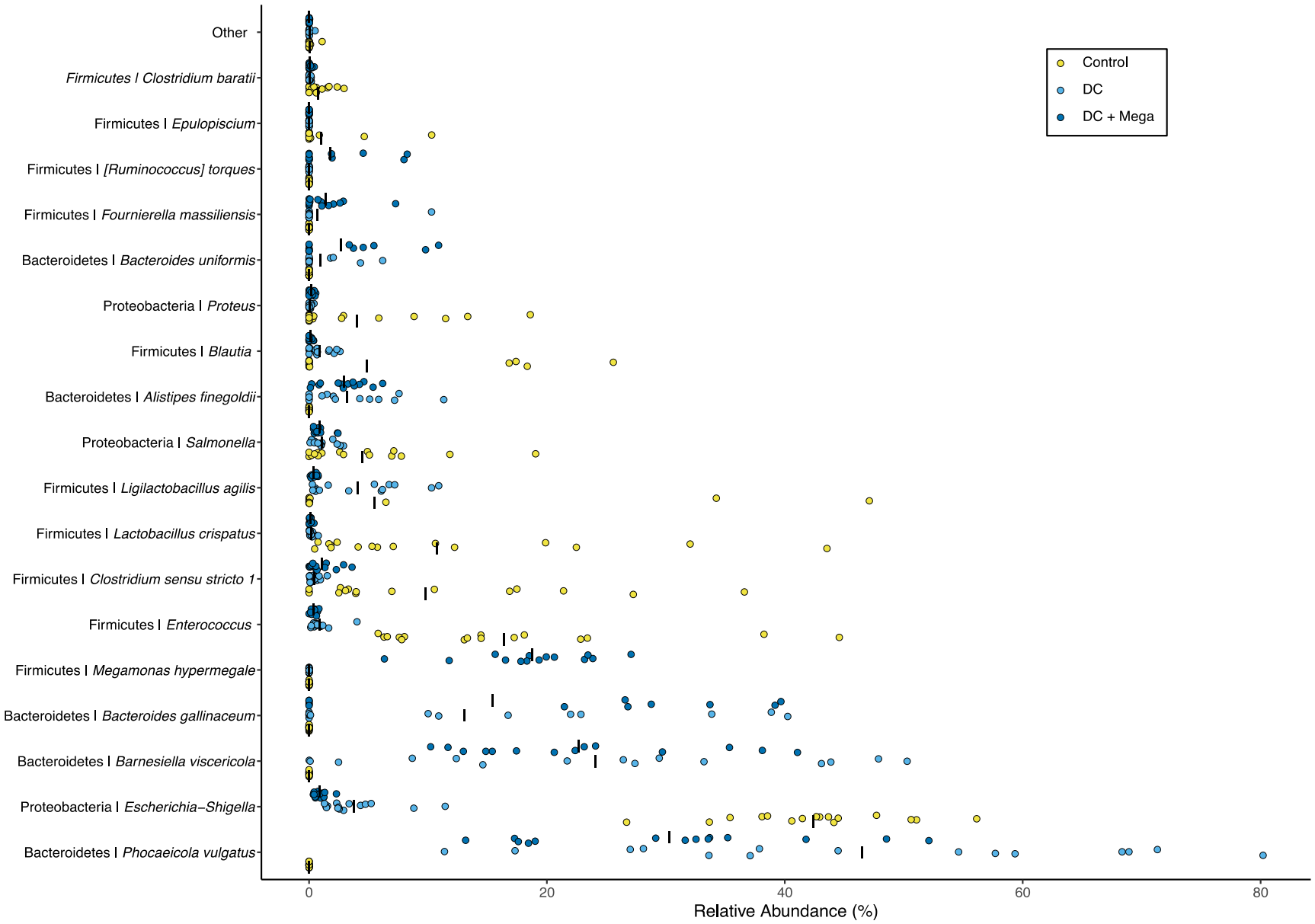

Other
Firmicutes | Clostridium baratii
Firmicutes | Epulopiscium
Firmicutes | [Ruminococcus] torques
Firmicutes | Fournierella massiliensis
Bacteroidetes | Bacteroides uniformis
Proteobacteria | Proteus
Firmicutes | Blautia
Bacteroidetes | Alistipes finegoldii
Proteobacteria | Salmonella
Firmicutes | Ligilactobacillus agilis
Firmicutes | Lactobacillus crispatus
Firmicutes | Clostridium sensu stricto 1
Firmicutes | Enterococcus
Firmicutes | Megamonas hypermegale
Bacteroidetes | Bacteroides gallinaceum
Bacteroidetes | Barnesiella viscericola
Proteobacteria | Escherichia−Shigella
Bacteroidetes | Phocaeicola vulgatus
Control
DC
DC + Mega
0
20
40
60
80
Relative Abundance (%)

Figure 5.5. The relative abundance of microbial taxa that were shown to be differentially abundant in the cecal microbiota of 14-day-old broilers from Control, DC, and DC + Mega treatments in E3 according to DESeq2 analysis. Dots represent the relative abundance of taxa in individual samples, and the bars represent the average relative abundance in each treatment. The relative abundance of taxa that were not differently abundant is shown as “Other”.

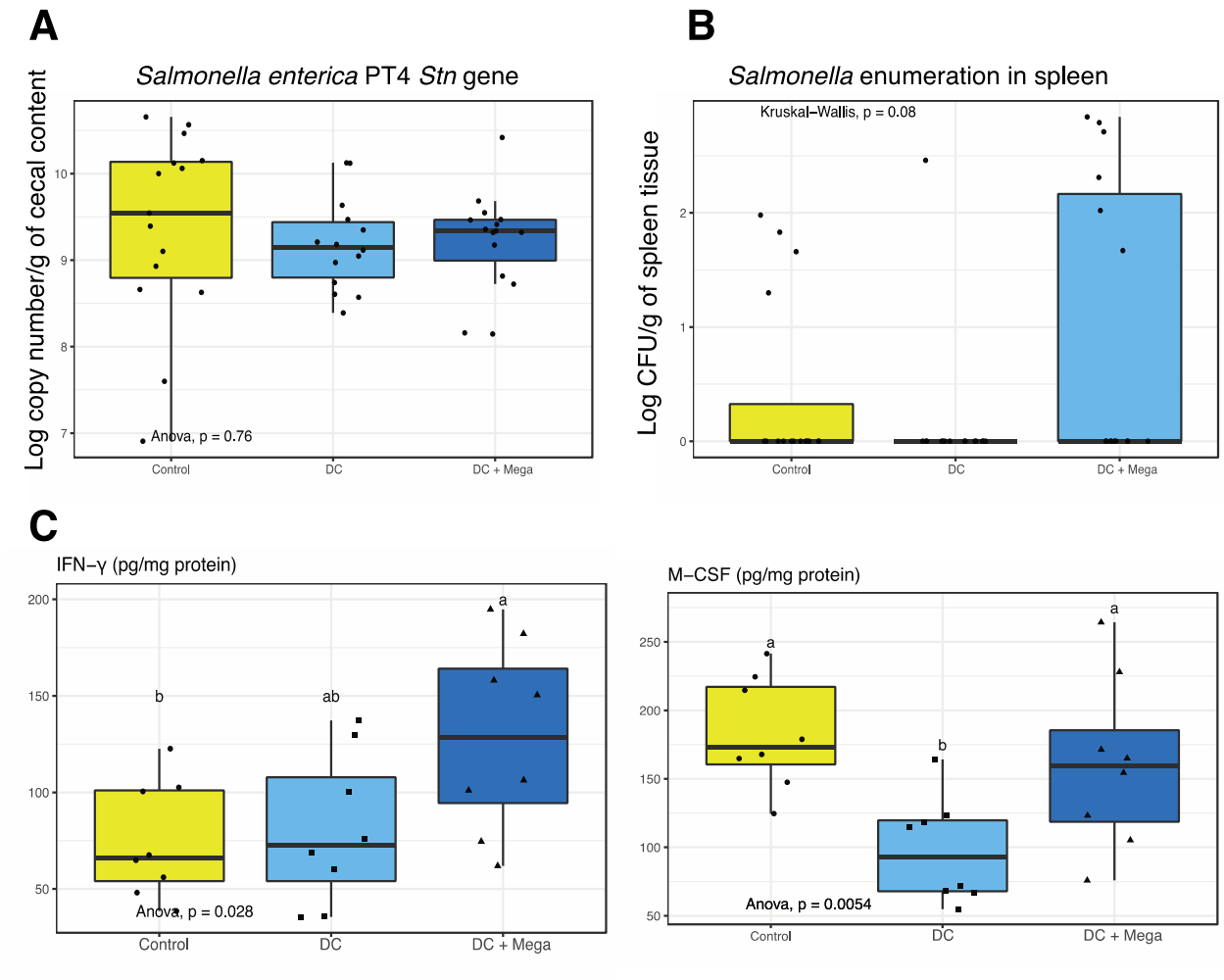


Figure 5.6. Dendrograms showing the effect of Control, DC and DC + Mega treatments in E3 on *Salmonella* colonization in **(A)** cecal contents based on *Stn* gene quantification by qPCR and in **(B)** spleen tissues based on culturing and enumeration methods. **(C)** Dendrograms showing the effects of treatments on the concentration of IFN-γ and M-CSF in the cecal tissues of 14-day-old broilers.

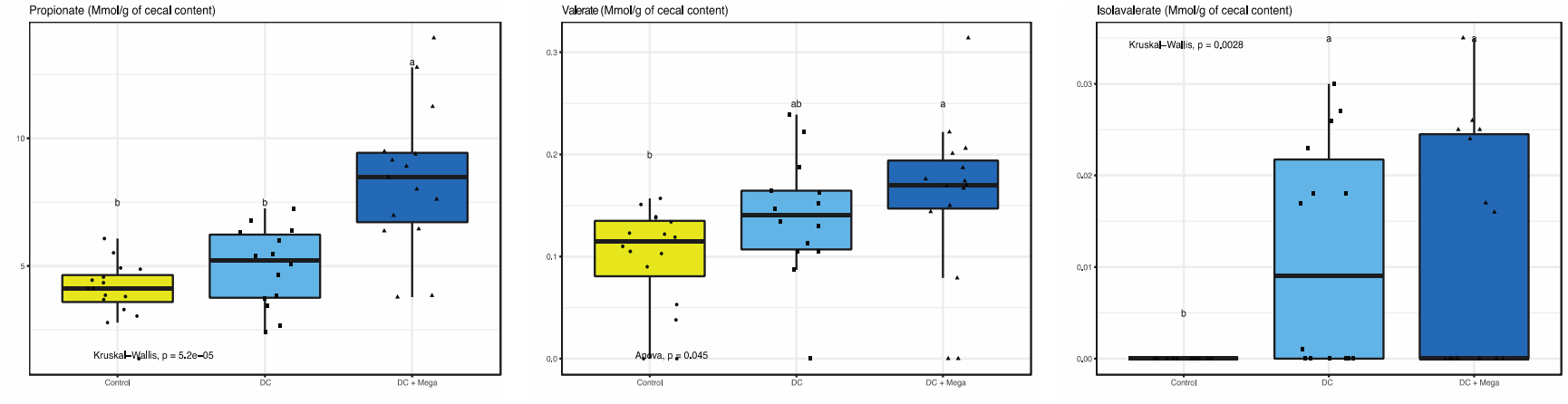


Figure 5.7. Dendrograms showing the effect of Control, DC and DC + Mega treatments in E3 on the concentration of short-chain fatty acids in the cecal content of 14-day-old broilers.

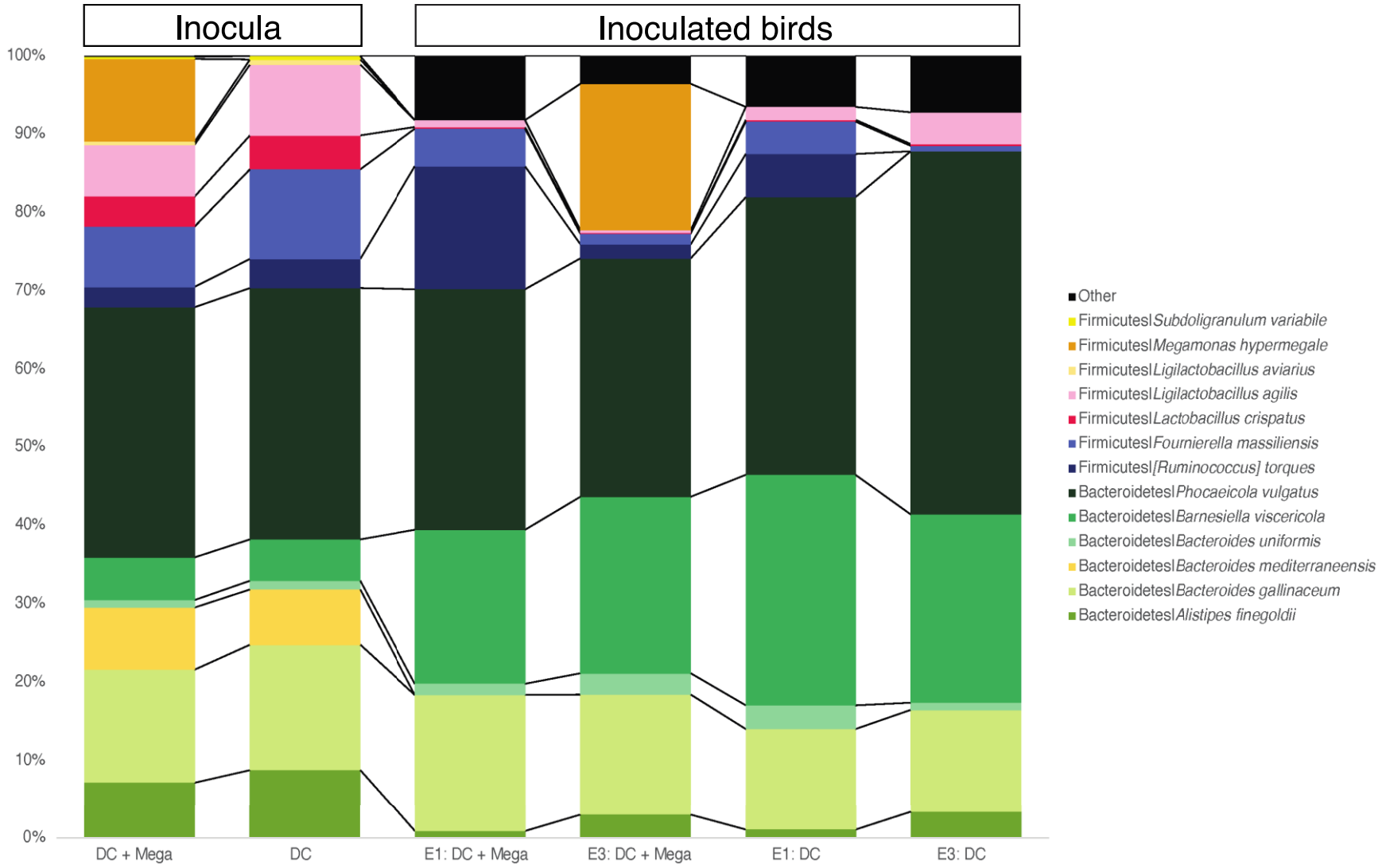

Inocula
Inoculated birds
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
DC + Mega
DC
E1: DC + Mega
E3: DC + Mega
E1: DC
E3: DC
Other
Firmicutes|Subdoligranulum variabile
Firmicutes|Megamonas hypermegale
Firmicutes|Ligilactobacillus aviarius
Firmicutes|Ligilactobacillus agilis
Firmicutes|Lactobacillus crispatus
Firmicutes|Fournierella massiliensis
Firmicutes|[Ruminococcus] torques
Bacteroidetes|Phocaeicola vulgatus
Bacteroidetes|Barnesiella viscericola
Bacteroidetes|Bacteroides uniformis
Bacteroidetes|Bacteroides mediterraneensis
Bacteroidetes|Bacteroides gallinaceum
Bacteroidetes|Alistipes finegoldii

Figure 5.8. Barplots showing the relative abundance of species included in the DC + Mega and DC inocula (first two bars) and the average relative abundance of these species in the inoculated birds in E1 and E3. Species present in the birds but not introduced by the inocula were combined as “Other” and are shown in black.

Table 5.1. Body weight (average ± SD, g) in all treatments across experiments.

Treatment	Control	Mega	DC	DC+Mega
2 week-old				
E1	409.5 ± 47.1	430.2 ± 61.5	415.7 ± 47.3	419.3 ± 48.7
E2	413.5 ± 52.2	408.9 ± 61.8	NA	NA
E3	367.5 ± 32.2	NA	368.1 ± 51.4	363.6 ± 49.2
1 week-old				
E1	144.9 ± 14.1	153.9 ± 13.9	152.1 ± 18.3	154.7 ± 19.9
E2	177.7 ± 25.5	183 ± 23.4	NA	NA
E3	137.6 ± 11.0	NA	139.9 ± 51.4	138.6 ± 14.8
At challenge				
E1	87 ± 6.5	93.2 ± 7.5	89.2 ± 8.8	92.6 ± 10
E2	92.0 ± 7.6	92.9 ± 10.0	NA	NA
E3	100 ± 6.6	NA	100.1 ± 11.0	96.0 ± 9.2
At arrival				
E1	48.6 ± 4.0	48.4 ± 5.9	48.5 ± 3.5	48.4 ± 5.6
E2	45.6 ± 3.3	46.0 ± 3.2		
E3	47.4 ± 3.1	NA	47.2 ± 3.0	47.7 ± 3.3

CHAPTER 6: GENERAL DISCUSSION

Intensive broiler production systems minimize the exposure of birds to maternal bacteria that are host-adapted and co-evolved with chickens in nature [1–3]. Most studies describing the microbiota composition of broilers have been conducted under high levels of sanitation and analyzed samples from artificially hatched chicks, which are likely to be colonized by environmental and human microbiota, rather than by chicken commensal bacteria [1–3]. Consequently, the microbiota of birds reared in experimental settings and in intensive production systems are unlikely to be representative of a chicken's natural microbiota.

Co-evolution is the ecological process in which two species impose selection pressure on each other and adapt their responses to improve fitness and prevent extinction [4]. Co-evolution shapes the relationship between hosts and their microbiomes in favor of beneficial partnerships [5]. The genetic repertoire of bacteria within the gut surpasses that of the host [6] and augments the host's metabolic capabilities. For example, bacteria within the gut can aid the digestion of non-starch polysaccharides from fibre, making oligo- and monosaccharides available to the host [7]. Moreover, bacterial fermentation of dietary fiber generates short-chain fatty acids, which can be used as energy substrates, regulate host metabolic and immune responses, and inhibit pathogen establishment [8]. Finally, inoculation of microbiota from wild individuals was shown to improve survival from infectious and tumorigenic challenges in captive animals [9]. Therefore, a healthy microbiota provides several advantages to the host. In turn, the host provides their microbiota with a sheltered environment that has relatively stable pH, oxygen levels, and temperature, in addition to a continuous flow of nutrients. Consequently, by hindering the colonization of broilers with chicken-adapted, co-evolved, natural microbiota, we might be damaging mutualistic and

commensal relationships that have been established and developed throughout the past 50,000 years.

6.1.1. Intensively raised broilers lack host-adapted bacterial lineages, especially Bacteroidetes

This thesis aimed to generate foundational knowledge and resources to harness the potential of the chicken microbiota in poultry production. The initial objectives were to identify differences between the cecal microbiota community of broilers in intensive and extensive production systems and identify bacteria that might be missing from broilers raised in intensive systems. We determined the variation in cecal microbiota composition between 35-day-old broilers reared in intensive and extensive commercial farm systems using 16S rRNA sequencing. Beta diversity analysis showed that the microbiota of broilers in both systems is different, and that the phylogenetic diversity was higher in broilers raised in extensive systems. Higher levels of phylogenetic diversity suggest that bacteria in the gut of extensively raised chickens are less related and more likely to perform distinguished metabolic functions within the gut [10]. Consistently, the predicted functional potential of the microbiota of extensively raised chickens was also higher than that of intensively raised birds. By performing a large array of metabolic functions, distantly related bacteria fill gut niches and make resources unavailable for other species, consequently hindering invading species' establishment [11]. This is particularly relevant for poultry production, as inoculating chicks with mature gut microbiota was consistently demonstrated to promote colonization resistance against Salmonella [12, 13].

In addition to the overall decrease in phylogenetic diversity, the microbiota of broilers in intensive systems was shown to present a reduced abundance of core bacteria that were ubiquitous in the gut of extensively raised broilers. The genera *Olsenella*, *Alistipes*, *Bacteroide*s,

Parabacteroides, *Megamonas*, and *Parasutterella* were particularly reduced in intensively raised broilers. These lineages of "missing bacteria" are anaerobic and lack mechanisms to survive in aerobic environments, and their reduction indicates that their dispersal amongst the host population has been affected by current production practices [3, 14].

Once established that broilers in intensive systems were missing core bacteria that could be found in extensively raised broilers, we aimed to identify bacteria that are effective colonizers of the broiler ceca by evaluating bacterial persistence in the ceca at 2 or 3 weeks after a single bacterial exposure in early life. To that end, we performed a series of experiments to evaluate the impact of different microbial preparations (cecal contents vs microbial cultures), inoculation strategies (gavage vs spray and cohousing), and the source of microbes in the inocula (obtained from extensively raised vs intensively raised chickens) on the cecal microbiota and physiological responses of broilers. The results demonstrated that day-old chicks exposed to cecal contents or microbial cultures were readily colonized by Bacteroidetes and presented a significantly higher abundance of *Alistipes*, *Bacteroides*, *Barnesiella*, *Mediterranea*, *Megamonas*, *Parabacteroides*, *Phascolarctobacterium*, and *Subdoligranulum* than control birds. These results were consistently observed using inocula harvested from different donors and introduced via gavage, spray, or cohousing methods.

Interestingly, by comparing the microbiota composition between birds that were experimentally inoculated and the birds in the control groups, we observed that some results were similar to what was observed when comparing broilers in extensive and intensive systems. Namely, the microbiota of broilers from extensive production systems and that of chicks in the inoculation treatments presented a higher phylogenetic diversity and higher abundance of Bacteroidetes, including *Alistipes*, *Bacteroides*, and *Parabacteroides*, in addition to *Megamonas*

than that of intensively raised broilers and control birds. These suggest that intensive systems hinder the colonization by a host-adapted, natural microbiota, and that, once introduced, the missing bacteria readily colonize the ceca.

This study also showed that the cecal microbiota of inoculated birds did not resemble that of the inoculum received. In fact, despite the distinguishing composition of the inocula introduced, all recipient birds presented a similar microbiota profile, which was dominated by Bacteroidetes, followed by Firmicutes, and Proteobacteria, at average relative abundances of 56%, 30% and 9%, respectively. As an example, birds inoculated with a microbial culture containing 47% Fusobacteria, 42% Firmicutes, and 8% Bacteroidetes presented a microbiota composition with less than 0.5% Fusobacteria, and was instead dominated by Bacteroidetes (51%), Firmicutes (30%) and Proteobacteria (11%). This is in agreement with previous research which found that germ-free mice inoculated with zebra-fish microbiota will shape the composition of the introduced inoculum so that the microbiota of the recipient mice still resembles that of a typical mouse, rather than a zebra-fish microbiota [15]. The results observed in the experimental birds suggest that chicks also shape the composition of the inoculum to resemble that of a typical chicken, which, as observed in the extensively raised broilers, is meant to be dominated by Bacteroidetes, Firmicutes and Proteobacteria.

In contrast to what was observed for recipient birds, the microbiota of control chicks was depleted of Bacteroidetes. Previous studies have shown that Bacteroidetes are good colonizers of the ceca; and that inocula containing limited and undetected levels of these bacteria still promoted Bacteroidetes expansion and dominance in the microbiota of inoculated chicks [14, 16–19]. It has been proposed that the absence or reduced levels of Bacteroidetes observed in broiler studies [20, 21] likely resulted from an absent/delayed exposure to chicken-adapted bacteria in combination

with housing conditions with high levels of sanitation. Bacteroidetes are likely transmitted from mother to offspring and the relative abundance of Bacteroidetes members can reach up to 90% in the ceca of broilers without causing adverse effects [2, 3]. Interestingly, when we compared the microbiota of broilers in commercial farms to that of experimental chicks, we found that the microbiota of commercial broilers clustered closer to the microbiota of the experimental control birds than to that of the inoculated birds. This suggests that microbiota development can be severely hindered in broilers reared in intensive production systems and that Bacteroidetes are particularly affected by stringent sanitation and biosecurity procedures.

Last, we evaluated the effects of inoculating newly hatched chicks with *Megamonas hypermegale* alone or in combination with a defined community (DC or DC + Mega) containing 12 bacterial strains isolated from chickens. The 12 strains included in the DC were *Alistipes finegoldi*, *Bacteroides uniformis*, *B. mediterraneensis*, *B. gallinaceum*, *Phocaeicola vulgatus*, *Barnesiella viscericola*, *Ligilactobacillus agilis*, *L. aviarius*, *L. crispatus*, [*Ruminococcus*] *torques*, *Subdoligranulum variable*, and *Fournierella massiliensis*. The strains that were introduced made up an average of 93% of the cecal microbiota of recipient birds at 14 days old. *P. vulgatus*, *B. viscericola* and *B. gallinaceum* were the most successful colonizers, and, together, made up an average 75% of the total community in inoculated birds. Consistently with the previous inoculation studies and to what was observed in extensively raised birds, we found that the proportion of Bacteroidetes was increased by microbial exposure.

In summary, it was shown that the cecal microbiota of broilers from intensive production systems present lower abundance of core microbes and putative functions compared to that of broilers from extensive systems. Some bacterial lineages that were reduced in intensive systems were shown to effectively colonize and become dominant in the microbiota of birds exposed to

complex or defined communities harvest from the chicken ceca. The most successful colonizers were strict anaerobes that are not capable to survive in aerobic environments and likely rely on host-adaptation mechanisms and close contact between individual hosts to disperse, namely *Alistipes*, *Bacteroides*, *Parabacteroides*, and *Megamonas*. Due to the biosafety practices employed in the poultry industry, which hinder the contact between newly hatched chicks and their parental microbiota, these lineages, in particular Bacteroidetes, are likely to be lost, reduced, or colonized with delay in commercial broilers, as previously indicated. Consequently, once introduced to the gut environment they can occupy available niches and efficiently engraft.

6.1.2. Major differences in microbiota composition led to relatively minor effects on host physiology

The inoculation of complex cecal communities or selected strains caused major differences between the cecal microbiota of control and inoculated birds; however, the observed effects on measured host responses were minor. Chicks inoculated with adult ceca contents via gavage showed lower ileal villus height crypt depth ratio, longer villi, and deeper crypts than control birds, suggesting an increase in cell renewal rate. In addition, these birds showed lower body weight (BW) than the control group at days 7 and 14 of age, but no significant differences in BW were observed on day 21. It is expected that birds exposed to a higher microbial load present a reduction in performance, due to an upregulation of immune responses which can divert nutrients from growth [22, 23]; however, when chicks were exposed to the DC, we observed no differences in BW compared to control chicks. The effects of bacterial exposure on BW are inconsistent across studies, ranging from improved [24, 25], not changed [18, 26–28], and reduced BW [29, 30]. However, historically, most studies that evaluated the introduction of complex communities and

the broiler responses to *Salmonella* infection had focused on *Salmonella* loads and did not report production performance data [24, 31–40].

The concentration of interferon (IFN)-α, IFN-γ, interleukin (IL)-2, IL-6, IL-10, IL-16, IL-21, macrophage inflammatory protein (MIP)-1b, MIP-3a, macrophage colony-stimulating factor (M-CSF), CCL5/regulated on activation, normal T cell expressed and secreted (RANTES), and vascular endothelial growth factor A (VEGF) were measured in the cecal tissues of experimental chicks. We found that IL-6 and IL-10 were higher in birds inoculated with complex communities compared to control groups, which suggests an activation of pro-inflammatory pathways and T-regulatory cell responses. Meanwhile, birds inoculated with defined communities showed higher IFN-γ concentrations compared to control birds. The production of IFN-γ by T-cells is stimulated by exposure to antigens [41] and is essential for effective infection clearance [42–46]. We also find that birds inoculated with DC community without *M. hypermegale* presented lower M-CSF, which is involved in monocyte differentiation, macrophage survival, and phagocytic activity [47]. *Salmonella* can cause systemic infection by invading macrophages and evading phagocytosis. Infected macrophages are then translocated to the spleen and liver, and *Salmonella* replicates in these organs [48, 49]. Interestingly, the lower M-CSF concentration in the ceca of DC colonized birds coincided with a lower spleen count of *Salmonella*, thus we speculated that the DC community reduced the rate of macrophage invasion and *Salmonella* systemic translocation. In addition, the *Salmonella* load in the gut of DC colonized birds was numerically reduced compared to the control and DC + Mega groups, indicating a potential avenue to be explored.

We also found that microbial inoculations with complex or defined communities led to an increase in the concentration of luminal propionate and valerate in the ceca compared to control birds. Bacterial fermentation in the gut leads to short-chain fatty acids production, mainly acetate,

butyrate, and propionate, which play a large array of functions within the gut environment and have major effects on host physiology [8]. The production of short-chain fatty acids is one of the proposed mechanisms by which bacteria can promote pathogen exclusion. It is proposed that short-chain fatty acids enter the bacterial cytoplasm and dissociate, causing acidification of the intracellular compartment and eliciting acid tolerance responses that compromise bacterial metabolic reactions and are energy-demanding [50].

Propionate is mainly produced from lactate and pyruvate by Bacteroidetes, Negativicutes, and *Clostridium* species [51]. There is scant information about valerate. Valerate is produced from ethanol, propionate and amino acids by *Clostridium* and methanogenic species [52–54]. Its role in chicken physiology is largely unexplored, although increased levels of propionate and valerate were reported in birds inoculated with cecal microbiota [39] and in week-old broilers harboring high Bacteroidetes abundance [55]. Another study indicated that propionate and valerate were positively correlated with *Bacteroides* and negatively correlated with *Salmonella* and *Escherichia-Shigella* abundance in challenged broilers [56], and these are in agreement with the trends observed in our inoculation studies.

In our experiments, propionate levels were higher in birds inoculated with complex microbial communities compared to chicks in the control group, which was linked to the increased abundance of Bacteroidetes, *Megamonas*, *Megasphaera*, and *Phascolactobacterium* (the latter three being Negativicutes) in inoculated chicks. Interestingly, in the experiment using defined communities, the group colonized with DC + Mega had a higher concentration of propionate than that of the DC-treated and the control groups. Since both the microbiota of DC and DC + Mega were enriched for Bacteroidetes, and only differed by *M. hypermegale* presence, the data suggests that *M. hypermegale* plays a major role as a propionate producer within the gut.

In summary, the exposure of chicks to bacterial inocula had moderate effects on cytokine concentration and increased propionate and valerate concentrations in the ceca. These were not linked to major changes in phenotype, for example, there were no differences in *Salmonella* load in challenged birds, no differences in whole blood bactericidal capacity, and no differences in final body weight. Nonetheless, we identified a knowledge gap regarding the effects of valerate and propionate on microbial communities and chicken physiology. Previous studies have shown that microbial cultures failed to protect chicks against *Salmonella* infection when the levels of propionate in the ceca of inoculated birds were low, thus suggesting propionate could promote disease resistance [57]. On the other hand, propionate supplementation failed to confer protection against *Salmonella* infection, suggesting that inhibition of *Salmonella* was due to other factors, other than higher propionate [58]. In addition, treatment of cultured Th1 cells with 0.5mM propionate was shown to promote IL-10 and IFN-γ production [59], which is compatible with the observed increased in IL-10 in birds inoculated with cecal contents and the increased IFN-γ in birds colonized with DC + Mega. These findings warrant further investigation on the effects of propionate in the luminal environment.

6.1.3. Commensal bacteria isolated in pure cultures are a valuable resource for basic and applied research

In this study, we generated a collection of chicken commensal bacteria containing 410 identified isolates in pure culture, spanning 6 phyla, and 87 species. Genomic characterization was performed on 24 isolates, from which 5 were found to be novel species. Similar culturing studies aiming at isolating poultry commensals in pure cultures have been performed. Ferrario et al., (2017) [60] have isolated 43 species; Medvecky et al. (2018) [61] isolated and genomic

characterized 133 strains, spanning 7 phyla and 59 species; Zenner et al. (2021) [62] cultured samples obtained from chickens of various ages, including one free-range layer, in six different media, and isolated 43 species. Interestingly, two studies failed to isolate Bacteroidetes [63, 64]. The studies that failed to isolate Bacteroidetes used samples collected from 31-day-old Ross broilers that were reared in fresh litter in experimental facilities [63], and from 7-day-old broilers reared in experimental farms [64]. The inability to culture Bacteroidetes could be resultant of the culturing strategies used, but also from the fact that Bacteroidetes were reduced or absent in these intensively reared birds. In fact, in the latter study, Bacteroidetes were detected in only 10 out of 30 samples used, and only 4 birds had Bacteroidetes at relative abundance higher than 5% [64]. If intensive production practices are causing the "disappearance of commensal microbes", these isolation efforts are valuable to preserve the native, host-adapted, commensal microbes.

6.2. LIMITATIONS

In this study, we used 16S rRNA sequencing to compare the cecal microbiota composition of intensively raised and extensively raised broilers in commercial farms in Alberta and found that intensive poultry production practices are linked to a reduction in the relative abundance of chicken commensal microbes that can be found in broilers in extensive rearing systems, namely *Olsenella*, *Alistipes*, *Bacteroides*, *Parabacteroides*, *Megamonas*, and *Parasutterella*. Throughout this study, we collected gut contents from chickens in different rearing systems and employed several culturing techniques to obtain mixed cultures and pure isolates of chicken commensal bacteria to be used in animal experiments. We created a culture collection containing at least 87 bacterial species, from which a subset of 24 isolates was characterized using whole-genome sequencing and indicated the isolation of 5 novel species. Next, we inoculated day-old chicks with cecal contents,

complex cultures, and selected isolates to determine the effects of an early-life inoculation with chicken commensals on microbiota development and host responses. We found that bacterial inoculations significantly affected the microbiota composition, however, relatively minor changes in host responses were observed.

Some of the limitations of this study are inherent to the use of 16S rRNA sequencing methods. For once, the data provided is compositional and does not necessarily reflect changes in the absolute abundance of taxa [65]. In addition, current methods to identify differences in relative abundance have low statistical power and higher rates of type 1 error (false-positive) [66]. In an attempt to reduce type 1 error, we performed differential abundance analyses using a combination of three methods; but the differences observed would still require to be validated by quantitative methods, such as qPCR. In addition, 16S rRNA sequencing has relatively lower accuracy in predicting the functional potential of the microbiota [67] and in performing taxonomy assignments compared to metagenomic sequencing [68]. Nonetheless, at least 96% of the sequencing reads in our dataset were assigned at least to the genus level, which was sufficient to indicate differences in the relative abundance of bacteria between groups, moreover, 16S rRNA is comparatively quicker, inexpensive, widely accepted in gut microbiota research, thus adequate for the objectives of this study.

Major limitations of our study include systematic biases, confounding factors, small sample size, data variability, and lack of replicability. For example, samples from broilers in intensive systems were collected during in-farm visits, whereas most of the samples collected from broilers in extensive systems were obtained from a provincially inspected slaughter plant. The sampling procedures in inoculation experiments were performed in a predetermined order that corresponded to the treatment groups. We recognize that these differences in sampling methods and time of

collection could have introduced systematic bias in our data [69]. In addition, we failed to account for sex differences as a confounding factor when analyzing microbiota composition and host responses. Male chickens present delayed humoral responses and lower survival rates compared to females, also, male microbiota was reported to be enriched for Bacteroidetes, *Megamonas*, *Phascolarctobacterium*, and *Megasphaera* compared to the microbiota of female broilers [70–72]. In the inoculation experiments, chicks were housed in isolators to avoid cross-contamination and reduce uncontrolled environmental factors, however, the number and size of the isolators limited the sample size and the duration of the experiments, as space in the isolators was insufficient to accommodate broilers older than 21-days. The failure to account for sex differences and the limited sample sizes were likely to have contributed to the variability observed in the host responses parameters evaluated. In subsequent studies, these can be mitigated by performing repeated trials and balancing for sex.

The defined community of isolates described in Chapter 4 was designed aiming to promote the establishment of a microbiota resembling that of a typical chicken. As we observed major differences in the relative abundances of Firmicutes and Bacteroidetes across different rearing systems, the DC community included 6 species from Bacteroidetes and 6 species from Firmicutes, with or without *M. hypermegale*. We did not include Proteobacteria since it is abundant in the microbiota of newly hatched chicks. A typical broiler microbiota, according to our previous data, would present a relative abundance of Bacteroidetes and Firmicutes ranging from 20% to 65%, and that of Proteobacteria ranging from 2% to 10%. Regardless of the presence of Firmicutes in the DC and of Proteobacteria in the baseline community, the microbiota of DC-treated birds was dominated by Bacteroidetes, reaching up to 96% in some individuals. In addition, some of the species included in the DC failed to colonize (*B. mediterraneensis*, *L. aviarius*, and *S. variabile*).

Therefore, we were not able to select the appropriate species to promote a typical chicken community, and this can be addressed in future studies by using different combinations of the isolates in our bacterial culture collection.

6.3. FUTURE DIRECTIONS

The culture collection of chicken commensal bacteria cultivated during this study presents an opportunity for the establishment of gnotobiotic chicken models. Gnotobiotic models, which are germ-free animals that have been colonized with a defined community of microorganisms, can limit the influence of microbiota variability in research trials [73]. Differences in initial microbiota composition affect the responses to experimental treatments and hinder the reproducibility of results [1, 2, 74]. Unlike germ-free animals, gnotobiotic models can recapitulate some of the phenotypes observed in conventional animals, while reducing the complexity and inter-individual variability of the microbiota [75, 76].

While gnotobiotic mouse models are well-established and widely used, gnotobiotic chicken models are less common and not standardized [40, 62, 77–79]. For the past 20 years, most studies on the broiler gut microbiota (including the present one) have been limited to describing changes in microbiota composition after numerous interventions and measuring a few host responses [80]. Therefore, we lack an understanding of microbiota function and the mechanisms driving the observed changes. The use of gnotobiotic chicken models allows research approaches that can help to elucidate the role of individual species within the microbiota community and their effects on chicken physiology.

Broadly, commensal bacteria can be systematically and comprehensively characterized using a combination of phenotypic and genotypic methods and screened for properties of interest,

such as antimicrobial resistance, pathogen inhibition, fiber fermentation, virulence factors, bacteriocin and short-chain fatty acids production using culture-dependent and culture-independent techniques [81]. Selected isolates can then be included in defined communities and evaluated using *in vitro* fermentation models, which allow the characterization of microbiota composition and function without the confounding effects of host-related factors [82]. Once defined communities that resemble that of a typical broiler and have functions of interest are established, these can be inoculated to germ-free chickens to evaluate the effects of these communities in vivo, as well as their impact on host-physiology.

These approaches offer exciting opportunities to advance our knowledge of the broiler microbiota beyond descriptive and correlational studies. These advancements can lead to significant progress in both basic and applied research. By gaining a comprehensive understanding of the function of individual bacteria and microbial communities, as well as their impact on host physiology, we have the potential to develop highly targeted probiotic products. These products can be precisely tailored to meet the specific production needs of farms, providing numerous opportunities to optimize broiler production and enhance disease resistance.

6.4. REFERENCES

1. Stanley D, Geier MS, Hughes RJ, Denman SE, Moore RJ. Highly variable microbiota development in the chicken gastrointestinal tract. PLoS One. 2013;8.

2. Rychlik I. Composition and function of chicken gut microbiota. Animals. 2020;10.

3. Kubasova T, Seidlerova Z, Rychlik I. Ecological adaptations of gut microbiota members and their consequences for use as a new generation of probiotics. International Journal of Molecular Sciences. 2021;22.

4. Janzen DH. When is it Coevolution? 1980.

5. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. Nature. 2017;548:43–51.

6. Henry LP, Bruijning M, Forsberg SKG, Ayroles JF. The microbiome extends host evolutionary potential. Nat Commun. 2021;12:5141.

7. Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, Weatherford J, et al. Glycan Foraging in Vivo by an Intestine-Adapted Bacterial Symbiont. Science (1979). 2005;307:1955–9.

8. Liu L, Li Q, Yang Y, Guo A. Biological Function of Short-Chain Fatty Acids and Its Regulation on Intestinal Health of Poultry. Front Vet Sci. 2021;8.

9. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, et al. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell. 2017;171:1015-1028.e13.

10. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489:220–30.

11. Stecher B, Chaffron S, Käppeli R, Hapfelmeier S, Freedrich S, Weber TC, et al. Like will to like: Abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathog. 2010;6.

12. Schneitz C. Competitive exclusion in poultry - 30 years of research. Food Control. 2005;16 8 SPEC. ISS.:657–67.

13. Kerr AK, Farrar AM, Waddell LA, Wilkins W, Wilhelm BJ, Bucher O, et al. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on Salmonella spp. prevalence and concentration in broiler chickens. Prev Vet Med. 2013;111:112–25.

14. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Gut anaerobes capable of chicken caecum colonisation. Microorganisms. 2019;7.

15. Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal Gut Microbiota Transplants from Zebrafish and Mice to Germ-free Recipients Reveal Host Habitat Selection. Cell. 2006;127:423–33.

16. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One. 2019;14.

17. Glendinning L, Chintoan-Uta C, Stevens MP, Watson M. Effect of cecal microbiota transplantation between different broiler breeds on the chick flora in the first week of life. Poult Sci. 2022;101:101624.

18. Poudel B, Shterzer N, Sbehat Y, Ben-Porat N, Rakover M, Tovy-Sharon R, et al. Characterizing the chicken gut colonization ability of a diverse group of bacteria. Poult Sci. 2022;101.

19. Ramírez GA, Richardson E, Clark J, Keshri J, Drechsler Y, Berrang ME, et al. Broiler chickens and early life programming: Microbiome transplant-induced cecal community dynamics and phenotypic effects. PLoS One. 2020;15 11 November.

20. Donaldson EE, Stanley D, Hughes RJ, Moore RJ. The time-course of broiler intestinal microbiota development after administration of cecal contents to incubating eggs. PeerJ. 2017;5:e3587.

21. Siegerstetter SC, Schmitz-Esser S, Magowan E, Wetzels SU, Zebeli Q, Lawlor PG, et al. Intestinal microbiota profiles associated with low and high residual feed intake in chickens across two geographical locations. PLoS One. 2017;12.

22. Klasing KC. Nutritional Modulation of Resistance to Infectious Diseases.

23. Klasing KC, Laãoerin DE, Peng RK, Fry AM. Immunologically Mediated Growth Depression in Chicks: Influence of Feed Intake, Corticosterone and Interleukin-11.

24. Corrier DE, Nisbet DJ, Scanlan CM, Hollister AG, Caldwell DJ, Thomas LA, et al. ENVIRONMENT AND HEALTH Treatment of Commercial Broiler Chickens with a Characterized Culture of Cecal Bacteria to Reduce Salmonellae Colonization 1. 1995.

25. Kayal A, Stanley D, Radovanovic A, Horyanto D, Van TTH, Bajagai YS. Controlled Intestinal Microbiota Colonisation in Broilers under the Industrial Production System. Animals. 2022;12.

26. Szott V, Reichelt B, Friese A, Roesler U. A Complex Competitive Exclusion Culture Reduces Campylobacter jejuni Colonization in Broiler Chickens at Slaughter Age In Vivo. Vet Sci. 2022;9.

27. Pottenger S, Watts A, Wedley A, Jopson S, Darby AC, Wigley P. Timing and delivery route effects of cecal microbiome transplants on Salmonella Typhimurium infections in chickens: potential for in-hatchery delivery of microbial interventions. Anim Microbiome. 2023;5.

28. Nakamura A, Ota Y, Mizukami A, Ito T, Ngwai YB, Adachi Y. Evaluation of Aviguard, a Commercial Competitive Exclusion Product for Efficacy and After-Effect on the Antibody Response of Chicks to Salmonella.

29. Torok VA, Hughes RJ, Ophel-Keller K, Ali M, MacAlpine R. Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult Sci. 2009;88:2474–81.

30. Śliżewska K, Markowiak-Kopeć P, Żbikowski A, Szeleszczuk P. The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. Sci Rep. 2020;10.

31. Nurmi E, Rantala M. New aspects of salmonella infection in broiler production. Nature. 1973;241:210–1.

32. Impey CS, Mead GC, George SM. Competitive Exclusion of Salmonellas from the Chick caecum Using a Defined Mixture of Bacterial Isolates from the Caecal Microflora of an Adult. 1982.

33. Stavric S, Gleeson TM, Blanchfield B, Pivnick H. Competitive Exclusion of Salmonella from Newly Hatched Chicks by Mixtures of Pure Bacterial Cultures Isolated from Fecal and Cecal Contents of Adult Birds. J Food Prot. 1985;48:778–83.

34. Gleeson TM, Stavric S, Blanchfield B. American Association of Avian Pathologists Allen Press Protection of Chicks against Salmonella Infection with a Mixture of Pure Cultures of Intestinal.

35. Corrier DE, David !, Nisbet J, Ii JAB, Hargis BM, Keith NK, et al. Dosage Titration of a Characterized Competitive Exclusion Culture To Inhibit Salmonella Colonization in Broiler Chickens during Growout t. 1998.

36. Stern NJ, Cox NA, Bailey JS, Berrang ME, Musgrove MT. Comparison of Mucosal Competitive Exclusion and Competitive Exclusion Treatment to Reduce Salmonella and Campylobacter spp. Colonization in Broiler Chickens.

37. Ferreira AJP, Ferreira CSA, Knobl T, Moreno AM, Bacarro MR, Chen M, et al. Comparison of Three Commercial Competitive-Exclusion Products for Controlling Salmonella Colonization of Broilers in Brazil. 2003.

38. Revolledo L, Ferreira CSA, Ferreira AJP. Prevention of Salmonella Typhimurium colonization and organ invasion by combination treatment in broiler chicks. Poult Sci. 2009;88:734–43.

39. Zaytsoff SJM, Montina T, Boras VF, Brassard J, Moote PE, Uwiera RRE, et al. Microbiota Transplantation in Day-Old Broiler Chickens Ameliorates Necrotic Enteritis via Modulation of the Intestinal Microbiota and Host Immune Responses. Pathogens. 2022;11.

40. Wongkuna S, Kumar R, Ghimire S, Mattiello Drescher S. Identification of a microbial sub-community from the feral chicken gut that reduces Salmonella colonization and improves gut health in a gnotobiotic chicken model. bIOrXiv. 2022.

41. Breed DGJ, Schetters TPM, Verhoeven NAP, Boot-Groenink A, Dorrestein J, Vermeulen AN. Vaccination against Eimeria tenella infection using a fraction of E. tenella sporozoites selected by the capacity to activate T cells.

42. Wang S, Fan Y, Brunham RC, Yang X. IFN-γ knockout mice show Th2-associated delayed-type hypersensitivity and the inflammatory cells fail to localize and control chlamydial infection. Eur J Immunol. 1999;29:3782–92.

43. Eckmann L, Kagnoff MF. Cytokines in host defense against Salmonella. 2001.

44. Han Y, Renu S, Patil V, Schrock J, Feliciano-Ruiz N, Selvaraj R, et al. Immune Response to Salmonella Enteritidis Infection in Broilers Immunized Orally With Chitosan-Based Salmonella Subunit Nanoparticle Vaccine. Front Immunol. 2020;11.

45. Bescucci DM, Montina T, Boras VF, Inglis GD. Infection by Salmonella enterica Serovar Typhimurium DT104 Modulates Immune Responses, the Metabolome, and the Function of the Enteric Microbiota in Neonatal Broiler Chickens. Pathogens. 2022;11.

46. Song B, Li P, Xu H, Wang Z, Yuan J, Zhang B, et al. Effects of rearing system and antibiotic treatment on immune function, gut microbiota and metabolites of broiler chickens. J Anim Sci Biotechnol. 2022;13.

47. Sebők C, Walmsley S, Tráj P, Mackei M, Vörösházi J, Petrilla J, et al. Immunomodulatory effects of chicken cathelicidin-2 on a primary hepatic cell co-culture model. PLoS One. 2022;17 10 October.

48. Chappell L, Kaiser P, Barrow P, Jones MA, Johnston C, Wigley P. The immunobiology of avian systemic salmonellosis. Vet Immunol Immunopathol. 2009;128:53–9.

49. Pham OH, McSorley SJ. Protective host immune responses to Salmonella infection. Future Microbiol. 2015;10:101–10.

50. Sun Y, O'Riordan MXD. Regulation of bacterial pathogenesis by intestinal short-chain fatty acids. In: Advances in Applied Microbiology. Academic Press Inc.; 2013. p. 93–118.

51. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, et al. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. ISME Journal. 2014;8:1323–35.

52. Bornstein BT, Barker HA. THE ENERGY METABOLISM OF CLOSTRIDIUM KLUYVERI AND THE SYNTHESIS OF FATTY ACIDS.

53. De Smit SM, De Leeuw KD, Buisman CJN, Strik DPBTB. Continuous n-valerate formation from propionate and methanol in an anaerobic chain elongation open-culture bioreactor. Biotechnol Biofuels. 2019;12.

54. Ma H, Liu H, Zhang L, Yang M, Fu B, Liu H. Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. Biotechnol Biofuels. 2017;10:137.

55. Fan Y, Ju T, Bhardwaj T, Korver DR, Willing BP. Week-Old Chicks with High Bacteroides Abundance Have Increased Short-Chain Fatty Acids and Reduced Markers of Gut Inflammation . Microbiol Spectr. 2023. https://doi.org/10.1128/spectrum.03616-22.

56. Thiam M, Wang Q, Barreto Sánchez AL, Zhang J, Ding J, Wang H, et al. Heterophil/Lymphocyte Ratio Level Modulates Salmonella Resistance, Cecal Microbiota Composition and Functional Capacity in Infected Chicken. Front Immunol. 2022;13.

57. Nisbet DJ, Corrier DE, Ricke SC, Hume ME, Byrd II JA, DeLoach JR. Cecal Propionic Acid as a Biological Indicator of the Early Establishment of a Microbial Ecosystem Inhibitory toSalmonellain Chicks. Anaerobe. 1996;2:345–50.

58. Hume ME, Corrier DE, Ambrus S, Hinton A, DeLoach JR. Effectiveness of dietary propionic acid in controlling Salmonella typhimurium colonization in broiler chicks. Avian Dis. 1993;37:1051–6.

59. Sun M, Wu W, Chen L, Yang W, Huang X, Ma C, et al. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. Nat Commun. 2018;9.

60. Ferrario C, Alessandri G, Mancabelli L, Gering E, Mangifesta M, Milani C, et al. Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses. Environ Microbiol. 2017;19:4771–83.

61. Medvecky M, Cejkova D, Polansky O, Karasova D, Kubasova T, Cizek A, et al. Whole genome sequencing and function prediction of 133 gut anaerobes isolated from chicken caecum in pure cultures. BMC Genomics. 2018;19.

62. Zenner C, Hitch TCA, Riedel T, Wortmann E, Tiede S, Buhl EM, et al. Early-Life Immune System Maturation in Chickens Using a Synthetic Community of Cultured Gut Bacteria. mSystems. 2021;6.

63. Moote PE, Zaytsoff SJM, Ortega Polo R, Abbott DW, Uwiera RRE, Douglas Inglis G. Application of culturomics to characterize diverse anaerobic bacteria from the gastrointestinal tract of broiler chickens in relation to environmental reservoirs. Can J Microbiol. 2020;66:288–302.

64. Duquenoy A, Ania M, Boucher N, Reynier F, Boucinha L, Andreoni C, et al. Caecal microbiota compositions from 7-day-old chicks reared in high-performance and low-performance industrial

farms and systematic culturomics to select strains with anti-Campylobacter activity. PLoS One. 2020;15:e0237541.

65. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. Front Microbiol. 2017;8.

66. Yang L, Chen J. A comprehensive evaluation of microbial differential abundance analysis methods: current status and potential solutions. Microbiome. 2022;10.

67. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38:685–8.

68. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013;31:814–21.

69. Jones J, Reinke SN, Ali A, Palmer DJ, Christophersen CT. Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations. Sci Rep. 2021;11.

70. Leitner G, Dan Heller E, Friedman A. Sex-related differences in immune response and survival rate of broiler chickens. Vet Immunol Immunopathol. 1989;21:249–60.

71. Cui L, Zhang X, Cheng R, Ansari AR, Elokil AA, Hu Y, et al. Sex differences in growth performance are related to cecal microbiota in chicken. Microb Pathog. 2021;150.

72. Lee KC, Kil DY, Sul WJ. Cecal microbiome divergence of broiler chickens by sex and body weight. Journal of Microbiology. 2017;55:939–45.

73. Brand MW, Wannemuehler MJ, Phillips GJ, Proctor A, Overstreet AM, Jergens AE, et al. The altered schaedler flora: Continued applications of a defined murine microbial community. ILAR J. 2015;56:169–78.

74. Ju T, Bourrie BCT, Forgie AJ, Pepin DM, Tollenaar S, Sergi CM, et al. The Gut Commensal Escherichia coli Aggravates High-Fat-Diet-Induced Obesity and Insulin Resistance in Mice. Appl Environ Microbiol. 2023;89.

75. Becker N, Kunath J, Loh G, Blaut M. Human intestinal microbiota: Characterization of a simplified and stable gnotobiotic rat model. Gut Microbes. 2011;2:25–33.

76. Darnaud M, De Vadder F, Bogeat P, Boucinha L, Bulteau A-L, Bunescu A, et al. A standardized gnotobiotic mouse model harboring a minimal 15-member mouse gut microbiota recapitulates SOPF/SPF phenotypes. Nat Commun. 2021;12:6686.

77. Baba E, Nagaishi S, Fukata T, Arakawa A. The Role of Intestinal Microflora on the Prevention of Salmonella Colonization in Gnotobiotic Chickens.

78. Thomas M, Wongkuna S, Ghimire S, Kumar R, Antony L, Doerner KC, et al. Gut Microbial Dynamics during Conventionalization of Germfree Chicken. mSphere. 2019;4.

79. Fuller R. Epithelial Attachment and Other Factors Controlling the Colonization of the Intestine of the Gnotobiotic Chicken by Lactobacilli. 1978.

80. Lee MD, Ipharraguerre IR, Arsenault RJ, Lyte M, Lyte JM, Humphrey B, et al. Informal nutrition symposium: leveraging the microbiome (and the metabolome) for poultry production. Poult Sci. 2022;101:101588.

81. Lagier JC, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, et al. Culturing the human microbiota and culturomics. Nature Reviews Microbiology. 2018;16:540–50.

82. Asare PT, Greppi A, Pennacchia A, Brenig K, Geirnaert A, Schwab C, et al. In vitro Modeling of Chicken Cecal Microbiota Ecology and Metabolism Using the PolyFermS Platform. Front Microbiol. 2021;12.

BIBLIOGRAPHY

Abbas, A.R., Modrusan, Z., Gong, Q., Zheng, Y., Sa, S.M., Ouyang, W., Danilenko, D.M., Valdez, P.A., Hu, Y., Ghilardi, N. and de Sauvage, F.J. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14: 282-289.

Abe, F., Ishibashi, N. and Shimamura, S. 1995. Effect of Administration of Bifidobacteria and Lactic Acid Bacteria to Newborn Calves and Piglets. J. Dairy Sci. 78:2838–2846.

Abreu, M.T. 2010. Toll-like receptor signaling in the intestinal epithelium: How bacterial recognition shapes intestinal function. Nat. Rev. Immunol. 10:131–143.

Ahmadizar, F., Vijverberg, S.J.H., Arets, H.G.M., de Boer, A., Turner, S., Devereux, G., Arabkhazaeli, A., Soares, P., Mukhopadhyay, S., Garssen, J., Palmer, C.N.A., de Jongste, J.C., Jaddoe, V.W.V., Duijts, L., van Meel, E.R., Kraneveld, A.D., and Maitland-van der Zee, A.H. 2017. Early life antibiotic use and the risk of asthma and asthma exacerbations in children. Pediatr. Allergy Immunol. 28:430–437.

Alberdi A, Aizpurua O, Bohmann K, Zepeda-Mendoza ML, Gilbert MTP. Do Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation? Trends in Ecology and Evolution. 2016;31:689–99.

Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, et al. CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res. 2020;48:D517–25.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic Local Alignment Search Tool. J Mol Biol. 1990;215:403–10.

Andreatti Filho, R.L., Marcos Sampaio, H., Rodrigues Barros, M., Gratão, P.R, and Cataneo, A. 2003. Use of cecal microflora cultured under aerobic or anaerobic conditions in the control of experimental infection of chicks with Salmonella Enteritidis. Vet. Microbiol. 92:237–244.

Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data . 2010.

Apajalahti J, Vienola K. Interaction between chicken intestinal microbiota and protein digestion. Anim Feed Sci Technol. 2016;221:323–30.

Ardissone, A.N., De La Cruz, D.M., Davis-Richardson, A.G., Rechcigl, K.T., Li, N.J., Drew, C.R., Murgas-Torrazza, R., Sharma, M.L., Hudak, E.W., Triplett, and Neu. J., 2014.

Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One. 9:1–8.

Arrieta MC, Stiemsma LT, Amenyogbe N, Brown E, Finlay B. The intestinal microbiome in early life: Health and disease. Frontiers in Immunology. 2014;5 AUG.

Asare PT, Greppi A, Pennacchia A, Brenig K, Geirnaert A, Schwab C, et al. In vitro Modeling of Chicken Cecal Microbiota Ecology and Metabolism Using the PolyFermS Platform. Front Microbiol. 2021;12.

Aziz RK, Bartels D, Best A, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: Rapid annotations using subsystems technology. BMC Genomics. 2008;9.

Baba E, Nagaishi S, Fukata T, Arakawa A. The Role of Intestinal Microflora on the Prevention of Salmonella Colonization in Gnotobiotic Chickens.

Bäckhed, F., H. Ding, T. Wang, L. V. Hooper, Y. K. Gou, A. Nagy, C. F. Semenkovich, and J. I. Gordon. 2004. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. U. S. A. 101:15718–15723. doi:10.1073/pnas.0407076101.

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A. and Gordon J.I., 2005. Host-Bacterial Mutualism in the Human Intestine. Science. 307:1915–1920.

Baht, M.I. and Kapila R. 2017. Dietary metabolites derived from gut microbiota:critical modulators of epigenetic changes in mammals. Nutr. Rev. 75(5):374-389.

Baldwin, S., R. J. Hughes, T. T. H. Van, R. J. Moore, and D. Stanley. 2018. At-hatch administration of probiotic to chickens can introduce beneficial changes in gut microbiota. PLoS One. 13:1–14. doi:10.1371/journal.pone.0194825.

Ballou, A. L., R. A. Ali, M. A. Mendoza, J. C. Ellis, H. M. Hassan, W. J. Croom, and M. D. Koci. 2016. Development of the chick microbiome: How early exposure influences future microbial diversity. Front. Vet. Sci. 3:1–12. doi:10.3389/fvets.2016.00002.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology. 2012;19:455–77.

Bar Shira, E. and Friedman, A. 2018. Innate immune functions of avian intestinal epithelial cells: response to bacterial stimuli and localization of responding cells in the developing avian digestive tract. PLoS One 13(7): e0200393. https://doi.org/10.1371/journal.pone.0200393.

Barbera P, Kozlov AM, Czech L, Morel B, Darriba D, Flouri T, et al. EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences. Systematic Biology. 2019;68:365–9.

Barnes EC, Impby CS. The Occurence and Properties of Uric Acid Decomposing Anaerobic Bacteria in the Avian Caecum. Journal of Applied Bacteriology. 1974;37(3):393-409.

Barnes EM, Carter EL, Lewis JD. Predicting Microbiome Function Across Space Is Confounded by Strain-Level Differences and Functional Redundancy Across Taxa. Front Microbiol. 2020;11.

Barnes, E. M., C. S. Impey, and BJ.H. Stevens, 1979. Factors affecting the incidence and anti-Salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg. Camb. 82:263-283. DOI: 10.1017/s0022172400025687

Bäumler, A.J. and Sperandio, V., 2016. Interactions between the microbiota and pathogenic bacteria in the gut. Nature. 535:85–93.

Bearson, S.M.D., Allen, H.K., Bearson, B.L., Looft, T., Brunelle, B.W., Kich, J.D., Tuggle, C. K., Bayles, D.O., Alt, D., Levine, U.Y. and Stanton, T.B. 2013. Profiling the gastrointestinal microbiota in response to Salmonella: Low versus high Salmonella shedding in the natural porcine host. Infect. Genet. Evol. 16:330–340.

Becker N, Kunath J, Loh G, Blaut M. Human intestinal microbiota: Characterization of a simplified and stable gnotobiotic rat model. Gut Microbes. 2011;2:25–33.

Belkaid, Y. and T. W. Hand. 2014. Role of the microbiota in immunity and inflammation. Cell. 157:121–141.

Bensch HM, Tolf C, Waldenström J, Lundin D, Zöttl M. Bacteroidetes to Firmicutes: captivity changes the gut microbiota composition and diversity in a social subterranean rodent. Anim Microbiome. 2023;5.

Berg, G., D. Rybakova, D. Fischer, T. Cernava, M.-C. C. Vergès, T. Charles, X. Chen, L. Cocolin, K. Eversole, G. H. Corral, M. Kazou, L. Kinkel, L. Lange, N. Lima, A. Loy, J. A. Macklin, E. Maguin, T. Mauchline, R. McClure, B. Mitter, M. Ryan, I. Sarand, H. Smidt, B. Schelkle, H. Roume, G. S. Kiran, J. Selvin, R. S. C. de Souza, L. van Overbeek, B. K. Singh, M. Wagner, A. Walsh, A. Sessitsch, and M. Schloter. 2020. Correction to: Microbiome definition re-visited: old concepts and new challenges. Microbiome. 8:1–22. doi:10.1186/s40168-020-00905-x.

Bergström, A., Skov, T.H., Bahl, M.I., Roager, H.M., Christensen, L.B., Ejlerskov, K.T., Mølgaard, Michaelsen K.F,. Licth T.R. 2014. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of large cohort of Danish infants. App. Environ. Microbiol. 80(9): 2889-2900.

Bescucci DM, Montina T, Boras VF, Inglis GD. Infection by Salmonella enterica Serovar Typhimurium DT104 Modulates Immune Responses, the Metabolome, and the Function of the Enteric Microbiota in Neonatal Broiler Chickens. Pathogens. 2022;11.

Besemer J, Borodovsky M. GeneMark: Web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res. 2005;33 SUPPL. 2.

Biagi E, Mengucci C, Barone M, Picone G, Lucchi A, Celi P, et al. Effects of Vitamin B2 Supplementation in Broilers Microbiota and Metabolome. Microorganisms. 2020;8:1134.

Bindari YR, Gerber PF. Centennial Review: Factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance. Poultry Science. 2022;101.

Bisanz JE. qiime2R: Importing QIIME2 artifacts and associated data into R sessions.

Blanchfield, B., S. Stavric, T. Gleeson, and H. Pivnick. 1984. Minimum intestinal inoculum for Nurmi cultures and a new method for determining competitive exclusion of Salmonella from chicks. J. Food Prot. 47:542–545. doi:10.4315/0362-028x-47.7.542.

Blaser, M. J. 2017. The theory of disappearing microbiota and the epidemics of chronic diseases. Nat. Rev. Immunol. 17:461–463.

Blaser, M. J., and Falkow, S., 2009. What are the consequences of the disappearing human microbiota? Nat. Rev. Microbiol. 7:887–894.

Bokulich, N. A., B. D. Kaehler, J. R. Rideout, M. Dillon, E. Bolyen, R. Knight, G. A. Huttley, and J. Gregory Caporaso. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 6:1–17. doi:10.1186/s40168-018-0470-z.

Bokulich, N.A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H.D., Lieber, A., Wu, F., Perez-Perez, G.I., Chen, Y., Schweizer, Zheng, X., Contreras, M., Dominguez-Bello, M.G. and Blaser M.J. 2016. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 8(343): 1-25.

Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7.

Bomba, L., Minuti, A., Moisá, S. J, Trevisi, E., Eufemi, E., Lizier, M., Chegdani, F. Lucchini, F., Rzepus, M., Prandini, A., Rossi, F., Mazza, R., Bertoni, R., Loor, J.J. and Ajmone-Marsan, P. 2014. Gut response induced by weaning in piglet features marked changes in immune and inflammatory response. Funct. Integr. Genomics. 14:657–671.

Bornstein BT, Barker HA. The energy metabolism of clostridium kluyveri and the synthesis of fatty acids.

Borrelli L, Coretti L, Dipineto L, Bovera F, Menna F, Chiariotti L, et al. Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. Sci Rep. 2017;7.

Brand MW, Wannemuehler MJ, Phillips GJ, Proctor A, Overstreet AM, Jergens AE, et al. The altered schaedler flora: Continued applications of a defined murine microbial community. ILAR J. 2015;56:169–78.

Breed DGJ, Schetters TPM, Verhoeven NAP, Boot-Groenink A, Dorrestein J, Vermeulen AN. Vaccination against Eimeria tenella infection using a fraction of E. tenella sporozoites selected by the capacity to activate T cells.

Brooks, J.P., Edwards, D.J., Harwich, M.D. et al. 2015. The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies. BMC Microbiol 15, 66 https://doi.org/10.1186/s12866-015-0351-6

Broom LJ, Kogut MH. The role of the gut microbiome in shaping the immune system of chickens. Veterinary Immunology and Immunopathology. 2018;204:44–51.

Caballero, S. and E. G. Pamer. 2015. Microbiota-Mediated Inflammation and Antimicrobial Defense in the Intestine. Ann. Rev. Imm. 33:227–256.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods. 13:581–583. doi:10.1038/nmeth.3869.

Canadian Integrated Program for Antimicrobial Resistance Surveillance.

Cario, E., G. Gerken, and D. K. Podolsky. 2007. Toll-Like Receptor 2 Controls Mucosal Inflammation by Regulating Epithelial Barrier Function. Gastroenterology. 132:1359–1374.

Castellano, P., Pérez Ibarreche, M., Blanco Massani, M., Fontana, C. and Vignolo, G. 2017. Strategies for Pathogen Biocontrol Using Lactic Acid Bacteria and Their Metabolites: A Focus on Meat Ecosystems and Industrial Environments. Microorganisms. 5:38.

Centers for Disease Control and Prevention (CDC). Available at https://www.cdc.gov/salmonella/index.html

Chang, J. Y., Antonopoulos, D.A., Kalra, A., Tonelli, A., Khalife, W.T., Schmidt, T.M. and V.B. Young. 2008. Decreased Diversity of the Fecal Microbiome in Recurrent Clostridium difficile –Associated Diarrhea. J. Infect. Dis. 197:435–438.

Chang, P. V., Hao, L., Offermanns, S. and Medzhitov, R. 2014. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc. Natl. Acad. Sci. 111:2247–2252.

Chappell L, Kaiser P, Barrow P, Jones MA, Johnston C, Wigley P. The immunobiology of avian systemic salmonellosis. Vet Immunol Immunopathol. 2009;128:53–9.

Cheled-Shoval SL, Gamage NSW, Amit-Romach E, Forder R, Marshal J, Van Kessel A, et al. Differences in intestinal mucin dynamics between germ-free and conventionally reared chickens after mannan-oligosaccharide supplementation. Poult Sci. 2014;93:636–44.

Chen, X., Xu, J., Ren, E., Su, Y. and Zhu, W. 2018. Co-occurrence of early gut colonization in neonatal piglets with microbiota in the maternal and surrounding delivery environments. Anaerobe 49:30-40.

Chen, Y., Song, T., Le Xiao Y., Wan, X., Yang, L., Li, J., Zeng, G., Fang, P., Wang, Z.Z. and Gao, R. 2018. Enhancement of immune response of piglets to PCV-2 vaccine by porcine IL-2 and fusion IL-4/6 gene entrapped in chitosan nanoparticles. Res. Vet. Sci. 117:224–232.

Chica Cardenas LA, Clavijo V, Vives M, Reyes A. Bacterial meta-analysis of chicken cecal microbiota. PeerJ. 2021 Jan 5;9:e10571. doi: 10.7717/peerj.10571. PMID: 33505795; PMCID: PMC7792525.

Chichlowski, M., Croom, J., McBride, B.W., Haveinstein, G.B., and Koci, M.D. 2007. Metabolic and Physiological Impact of Probiotics or Direct-Fed-Microbials on Poultry: a brief review of currently knowledge. Int. J. Poult. Sci. 6:694–704.

Chintoan-Uta C, Wisedchanwet T, Glendinning L, Bremner A, Psifidi A, Vervelde L, et al. Role of Cecal Microbiota in the Differential Resistance of Inbred Chicken Lines to Colonization by Campylobacter jejuni. 2020. https://doi.org/10.1128/AEM.

Cho, I. and Blaser, M. J. 2012. The human microbiome: At the interface of health and disease. Nat. Rev. Genet. 13:260–270.

Cho, I., Yamanishi, S., Cox, L., Methé, B.A., Zavadil, J., Kelvin, L., Gao, Z., Mahana, D., Raju K., Teitle, I., Li, H., Alekseyenko, A.V., and Blaser, M.J. 2012. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 488 (7413):621-626.

Chung, H., Pamp, S.J., Hill, J.A., Surana, N.K., Sanna, M., Troy, E.B., Reading, N.C., Villablanca, E.J., Wang, S., Mora, J.R., Umesaki, Y., Mathis, D., Benoist, C., David, A. and Kasper, D.L.2012. Gut immune maturation depends on colonization with a host-specific microbiota. Cell. 149:1578–1593.

Coates ME, Fuller R, Harrison GF, Lev M, Suffolk SF. A comparision of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. British Journal of Nutrition. 1963;17:141–50.

Coble DJ, Redmond SB, Hale B, Lamont SJ. 2011. Distinct lines of chickens express different splenic cytokine profiles in response to Salmonella Enteritidis challenge. Poult Sci. (8):1659-63. doi: 10.3382/ps.2010-01279.

Colditz, I. G. 2002. Effects of the immune system on metabolism: Implications for production and disease resistance in livestock. Livest. Prod. Sci. 75:257–268.

Colombino E, Biasato I, Ferrocino I, Oddon SB, Caimi C, Gariglio M, et al. Effect of insect live larvae as environmental enrichment on poultry gut health: Gut mucin composition, microbiota and local immune response evaluation. Animals. 2021;11.

Colson, V., Orgeur, P., Foury, A. and Mormède, P. 2006. Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. Appl. Anim. Behav. Sci. 98:70–88.

Corcoran BM, Stanton C, Fitzgerald GF, Ross RP. Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. Appl Environ Microbiol. 2005;71:3060–7.

Corrier DE, David, Nisbet J, Ii JAB, Hargis BM, Keith NK, et al. Dosage Titration of a Characterized Competitive Exclusion Culture To Inhibit Salmonella Colonization in Broiler Chickens during Growout t. 1998.

Corrier DE, Hinton A, Hargis B, Deloach JR. Effect of Used Litter from Floor Pens of Adult Broilers on Salmonella Colonization of Broiler.

Corrier DE, Nisbet DJ, Hollister AG, Scanlan CM, Hargis BM, Deloach JR. Development of Defined Cultures of Indigenous Cecal Bacteria to Control Salmonellosis in Broiler Chicks 1.

Corrier DE, Nisbet DJ, Scanlan CM, Hollister AG, Caldwell DJ, Thomas LA, et al. ENVIRONMENT AND HEALTH Treatment of Commercial Broiler Chickens with a Characterized Culture of Cecal Bacteria to Reduce Salmonellae Colonization 1. 1995.

Corrier DE, Nisbet DJ, Scanlan CM, Hollister AG, Deloach JR. Environment and Health Control of Salmonella typhimurium Colonization in Broiler Chicks with a Continuous-Flow Characterized Mixed Culture of Cecal Bacteria 1.

Corrier, A. D. E., A. G. Hollister, D. J. Nisbet, C. M. Scanlan, and R. C. Beier. 1994. Competitive exclusion of Salmonella enteritidis in Leghorn Chicks: Comparison of treatment by crop gavage, drinking water, spray, or lyophilized alginate beads Avian Dis. 38:297–303

Corrier, D.E., Hinton, A.A., and Hargis, A.B.B. 2016. Effect of Used Litter from Floor Pens of Adult Broilers on Salmonella Colonization of Broiler Chicks. Avian Diseases. 36:897–902.

Costello, E. K., Stagaman, K., Dethlefsen L., Bohannan, B.J.M. and Relman D. 2012. The Application of Ecological Theory Toward and Understanding of the Human Microbiome. Science. 336:1255–1262.

Cowieson AJ. Comparative biology of germ-free and conventional poultry. Poultry Science. 2022;101.

Cox, L.M., Yamanishi, S., Sohn, J., Alekseyenko, A.V., Leung, J.M., Cho, I., Kim, S.G., Li, H., Gao, Z., Mahana, D., Zárate Rodriguez, J.G., Rogers, A.B., Robine, N., Loke, P., Blaser,

M.J. 2014. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 154(4): 705-721

Cressman MD, Yu Z, Nelson MC, Moeller SJ, Lilburn MS, Zerby HN. 2010. Interrelations between the microbiotas in the litter and in the intestines of commercial broiler chickens. Appl Environ Microbiol. 76(19):6572-82. doi: 10.1128/AEM.00180-10

Crisol-Martínez, E., Stanley, D., Geier, M.S, Hughes, R.J. and R. J. Moore. 2017. Understanding the mechanisms of zinc bacitracin and avilamycin on animal production: linking gut microbiota and growth performance in chickens. Appl. Microbiol. Biotechnol. 101:4547–4559.

Cui L, Zhang X, Cheng R, Ansari AR, Elokil AA, Hu Y, et al. Sex differences in growth performance are related to cecal microbiota in chicken. Microb Pathog. 2021;150.

Cui, Y., Wang, S. Liu, R. Sun, Y. Zhou, and Li, Y. 2017. Age-related variations in intestinal microflora of free-range and caged hens. Front. Microbiol. 8:1–10.

D'Eath, R.B.D., and Turner, S.P., 2009. The Welfare of Pigs. Available from: http://link.springer.com/10.1007/978-1-4020-8909-1

Dallas JW, Warne RW. Captivity and Animal Microbiomes: Potential Roles of Microbiota for Influencing Animal Conservation. Microbial Ecology. 2022.

Dame-Korevaar A, Kers JG, van der Goot J, Velkers FC, Ceccarelli D, Mevius DJ, et al. Competitive Exclusion Prevents Colonization and Compartmentalization Reduces Transmission of ESBL-Producing Escherichia coli in Broilers. Front Microbiol. 2020;11.

Darnaud, M., F. De Vadder, P. Bogeat, L. Boucinha, A. L. Bulteau, A. Bunescu, C. Couturier, A. Delgado, H. Dugua, C. Elie, A. Mathieu, T. Novotná, D. A. Ouattara, S. Planel, A. Saliou, D. Šrůtková, J. Yansouni, B. Stecher, M. Schwarzer, F. Leulier, and A. Tamellini. 2021. A standardized gnotobiotic mouse model harboring a minimal 15-member mouse gut microbiota recapitulates SOPF/SPF phenotypes. Nat. Commun. 12:1–21. doi:10.1038/s41467-021-26963-9.

Darwish, N., Shao, J., Schreier, L.L. et al. 2021. Choice of 16S ribosomal RNA primers affects the microbiome analysis in chicken ceca. Sci Rep 11, 11848. https://doi.org/10.1038/s41598-021-91387-w

Das B, Ghosh TS, Kedia S, Rampal R, Saxena S, Bag S, et al. Analysis of the gut microbiome of rural and urban healthy indians living in sea level and high altitude areas. Sci Rep. 2018;8.

Das Q, Shay J, Gauthier M, Yin X, Hasted T-L, Ross K, et al. Effects of Vaccination Against Coccidiosis on Gut Microbiota and Immunity in Broiler Fed Bacitracin and Berry Pomace. Front Immunol. 2021;12.

Dascalu S, Preston SG, Dixon RJ, Flammer PG, Fiddaman S, Boyd A, et al. The influences of microbial colonisation and germ-free status on the chicken TCRβ repertoire. Front Immunol. 2023;13.

De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010;107:14691–6.

De Passillè, A., Pelletier, G., Ménard, J., and Morisset, J. 1989. Relationships of weight gain and behavior to digestive organ weight and enzyme activities in piglets. J. Anim. Sci., 67:2921-2929.

De Smit SM, De Leeuw KD, Buisman CJN, Strik DPBTB. Continuous n-valerate formation from propionate and methanol in an anaerobic chain elongation open-culture bioreactor. Biotechnol Biofuels. 2019;12.

Dec M, Puchalski A, Urban-Chmiel R, Wernicki A. Screening of Lactobacillus strains of domestic goose origin against bacterial poultry pathogens for use as probiotics. Poult Sci. 2014;93:2464–72.

DeFronzo, R.A., Ferrannini, E., Groop, L., Henry, R.R., Herman, W.H., Holst, J.J, Hu, F.B., Kahn, C.R., Raz I., Shulman, G.I., Simonson, D.C., Testa, M.A and, Weiss R. 2015. Type 2 diabetes. Nat. Rev. 15019: 1-22.

Degruttola AK, Low D, Mizoguchi A, Mizoguchi E. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis. 2016;22:1137–50.

Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on humang-microbe mutualism and disease. Nature. 2007;449:811–8.

Dibner JJ, Richards JD. Antibiotic Growth Promoters in Agriculture: History and Mode of Action.

Donaldson, E.E., Stanley, D., Hughes, R.J. and Moore, R.J. 2017. The time-course of broiler intestinal microbiota development after administration of cecal contents to incubating eggs. PeerJ. 5:e3587.

Dong, S., B. H. Zeng, L. Hu, Y. Zhang, J. Xiong, J. Deng, L. Huang, Z. L. Liao, J. Wang, H. Wei, and X. Fang. 2021. Effect of a humanized diet profile on colonization efficiency and gut microbial diversity in human flora-associated mice. Front. Nutr. 8:1–14. doi:10.3389/fnut.2021.633738.

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol. 2020;38:685–8.

Drula E, Garron M-L, Dogan S, Lombard V, Henrissat B, Terrapon N. The carbohydrate-active enzyme database: functions and literature. Nucleic Acids Res. 2022;50:D571–7.

Duar, R.M., Burkey, T.E., Fernando, S.C., Wenzel, C.Q., Tasseva, G., Lin, X.B., Blom, J., Peterson, D.A., Walter, J., Szymanski, C.M. and Frese, S.A. 2017. Experimental evaluation of host adaptation of Lactobacillus reuteri to different vertebrate species. Appl. Env. Microb. 83:e0013-17

Duquenoy A, Ania M, Boucher N, Reynier F, Boucinha L, Andreoni C, et al. Caecal microbiota compositions from 7-day-old chicks reared in high-performance and low-performance industrial farms and systematic culturomics to select strains with anti-Campylobacter activity. PLoS One. 2020;15:e0237541.

Eckmann L, Kagnoff MF. Cytokines in host defense against Salmonella. 2001.

Eeckhaut V, Wang J, Van Parys A, Haesebrouck F, Joossens M, Falony G, et al. The probiotic butyricicoccus pullicaecorum reduces feed conversion and protects from potentially harmful intestinal microorganisms and necrotic enteritis in broilers. Front Microbiol. 2016;7 SEP.

Ella Barnes BM, Impey CS, H Stevens A BJ. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. 1979.

Elsherif HMR, Orabi A, Hassan HMA, Samy A. 2022. Sodium formate, acetate, and propionate as effective feed additives in broiler diets to enhance productive performance, blood biochemical, immunological status and gut integrity. Adv. Anim. Vet. Sci. 10(6):1414-1422. https://dx.doi.org/10.17582/journal.aavs/2022/10.6.1414.1422

Fagundes, C.T., Amaral, F.A., Vieira, A.T., Soares, A.C., Pinho, V., Nicoli J.R., Vieira, L.Q., Teixeira, M.M. and Souza, D.G. 2012. Transient TLR Activation Restores Inflammatory Response and Ability To Control Pulmonary Bacterial Infection in Germfree Mice. J. Immunol. 188:1411–1420.

Fan, Y., Ju, T., Bhardwaj, T., Korver, D. R, Willing, B. P. 2023 Week-old chicks with high Bacteroides abundance have increased short-chain fatty acids and reduced markers of gut inflammation. Microbiology Spectrum 10.1128/spectrum.03616-22 DOI: https://doi.org/10.1128/spectrum.03616-22

Farkas V, Csitári G, Menyhárt L, Such N, Pál L, Husvéth F, et al. Microbiota Composition of Mucosa and Interactions between the Microbes of the Different Gut Segments Could Be a Factor to Modulate the Growth Rate of Broiler Chickens. Animals. 2022;12.

Fasina, Y. O., Holt, P.S., Moran, E.T., Moore, R.W., Conner, D. E., McKee. S.R. 2008. Intestinal cytokine response of commercial source broiler chicks to Salmonella Typhimurium infection. Poult. Sci. 87:1335-1346. doi: https://doi.org/10.3382/ps.2007-00526.

Feng Y, Zhang M, Liu Y, Yang X, Wei F, Jin X, et al. Quantitative microbiome profiling reveals the developmental trajectory of the chicken gut microbiota and its connection to host metabolism. iMeta. 2023. https://doi.org/10.1002/imt2.105.

Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. ANOVA-Like Differential Expression (ALDEx) Analysis for Mixed Population RNA-Seq. PLoS One. 2013;8

Ferrario, C., Alessandri, G., Mancabelli, L., Gering, E., Mangifesta, M., Milani, C., Lugli, G.A., Viappiani, A., Duranti, S., Turroni, F., Ossiprandi, M.C., Hiyashi, R., Mackie, R., van Sinderen, D. and Ventura, M. 2017. Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses. Environ Microbiol, 19: 4771-4783. https://doi-org/10.1111/1462-2920.13943

Ferreira AJP, Ferreira CSA, Knobl T, Moreno AM, Bacarro MR, Chen M, et al. Comparison of Three Commercial Competitive-Exclusion Products for Controlling Salmonella Colonization of Broilers in Brazil. 2003.

Ferro PJ, Swaggerty CL, Kaiser P, Pevzner IY, Kogut MH. 2004. Heterophils isolated from chickens resistant to extra-intestinal Salmonella enteritidis infection express higher levels of pro-inflammatory cytokine mRNA following infection than heterophils from

susceptible chickens. Epidemiol Infect. 132(6):1029-37. doi: 10.1017/s0950268804002687.

Foditsch, C., Van Vleck Pereira, R., Ganda, E.K., Gomez, M.S., Marques, E.C., Santin T. and Bicalho, R.C. 2015. Oral administration of Faecalibacterium prausnitzii decreased the incidence of severe diarrhea and related mortality rate and increased weight gain in preweaned dairy heifers. PLoS One. 10:1–17.

Forder, R.E.A., Howarth, G.S., Tivey, D.R. and Hughes, R.J. 2007. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. Poult. Sci. 86:2396–2403.

Foster, K. R., J. Schluter, K. Z. Coyte, and S. Rakoff-Nahoum. 2017. The evolution of the host microbiome as an ecosystem on a leash. Nature. 548:43–51. doi:10.1038/nature23292.

Frese SA, MacKenzie DA, Peterson DA, Schmaltz R, Fangman T, Zhou Y, et al. Molecular Characterization of Host-Specific Biofilm Formation in a Vertebrate Gut Symbiont. PLoS Genet. 2013;9:e1004057.

Frese, S.A, Hutkins, R.W. and Walter, J. 2012. Comparison of the Colonization Ability of Autochthonous and Allochthonous Strains of Lactobacilli in the Human Gastrointestinal Tract. Adv. Microbiol. 02:399–409.

Fu, Y., J. Hu, M. A. Erasmus, T. A. Johnson, and H. wei Cheng. 2022. Effects of early-life cecal microbiota transplantation from divergently selected inbred chicken lines on growth, gut serotonin, and immune parameters in recipient chickens. Poult. Sci. 101:1–11. doi:10.1016/j.psj.2022.101925.

Fujimura, K.E., Sitarik, A.R., Havstad, S., Panzer, A.R., Boushey, H.A., Lukacs, N.W., Wegienka, G.R., Woodcroft, K.J., Zoratti, E.M., Ownby, D.R., Levin, A.M., Johnson, C.C. and Lynch, S.V. 2015. Development of childhood atopy is associated with early-life gut microbial enterotypes. Am J Respir Crit Care Med. 191:2186.

Fuller R. Epithelial Attachment and Other Factors Controlling the Colonization of the Intestine of the Gnotobiotic Chicken by Lactobacilli. 1978.

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., Takahashi, M., Fukuda, N.N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, J.M, Topping, D.L., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K.,

Hase, K. and Ohno H. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 504:446–450.

Furuse M, Okumura J-I. REVIEW Nutritional and physiological characteristics in germ-free chickens. 1994.

Furuse M, Yokota H. Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. British Journal of Nutrition. 1984;51:255.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., and Hinton, J.C.2006.Butyrate specifically down-regulates Salmonella Pathogenicity Island 1 Gene Expression. App. Environ. Microbiol. 72:946–949.

Gao K., Pi Y., Peng Y., Mu C.L. Zhu W.Y. 2018. Time-course responses of ileal and fecal microbiota and metabolite profiles to antibiotics in cannulated pigs. Appl. Microbiol. and Biotech. 102:1189-1199

García-Amado M, Michelangeli F, Gueneau P, Perez M, Domínguez-Bello M. Bacterial detoxification of saponins in the crop of the avian foregut fermenter <i>Opisthocomus hoazin</i> J Anim Feed Sci. 2007;16 Suppl. 2:82–5.

Geirnaert A, Steyaert A, Eeckhaut V, Debruyne B, Arends JBA, Van Immerseel F, et al. Butyricicoccus pullicaecorum, a butyrate producer with probiotic potential, is intrinsically tolerant to stomach and small intestine conditions. Anaerobe. 2014;30:70–4.

Geissinger O, Herlemann DPR, Morschel E, Maier UG, Brune A. The ultramicrobacterium "Elusimicrobium minutum" gen. nov., sp. nov., the first cultivated representative of the termite group 1 phylum. Appl Environ Microbiol. 2009;75:2831–40.

Ghebremicael, S. B., J. R. Hasenstein, and S. J. Lamont. 2008. Association of interleukin-10 cluster genes and Salmonella response in the chicken. Poult. Sci. 87:22–26. doi:10.3382/ps.2007-00259.

Gilbert, M., Xiao, X., and Robinson, T. P. 2018. Intensifying poultry production systems and the emergence of avian influenza in China: a "One Health/Ecohealth" epitome. Arch. Pub. Health. 75:48.

Gleeson TM, Stavric S, Blanchfield B. Protection of Chicks against Salmonella Infection with a Mixture of Pure Cultures of Intestinal Bacteria. Avian Dis. 1989;33:636.

Glendinning, L., C. Chintoan-Uta, M. P. Stevens, and M. Watson. 2022. Effect of cecal microbiota transplantation between different broiler breeds on the chick flora in the first week of life. Poult. Sci. 101:101624. doi:10.1016/j.psj.2021.101624.

Gloor GB, Macklaim JM, Pawlowsky-Glahn V and Egozcue JJ. 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. Front. Microbiol. 8:2224. doi: 10.3389/fmicb.2017.02224

Gomes, A.M.P. and F.X.Malcata. 1999. Bifidobacterium spp. and Lactobacillus acidophilus: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci. Technol. 10:139–157.

Gong Y, Yang H, Wang X, Xia W, Lv W, Xiao Y and Zou X. 2019. Early Intervention With Cecal Fermentation Broth Regulates the Colonization and Development of Gut Microbiota in Broiler Chickens. Front. Microbiol. 10:1422. doi: 10.3389/fmicb.2019.01422

Greene G, Koolman L, Whyte P, Burgess C, Bolton D. The Gut Microbiota of Broilers Reared with and without Antibiotic Treatment. Microorganisms. 2023;11:876.

Gresse, R., Chaucheyras-Durand, F., Fleury, M.A., Van de Wiele, T., Forano, E. and S. Blanquet-Diot, 2017. Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol. 25: 851-873.

Guillemet R., Hamard, A., Quesnel, H., Père, M.C., Etienne, M., Dourmad, J.Y. and Meunier-Salaün M.C. 2007. Dietary fibre for gestating sows: effects on parturition progress, behaviour, litter and sow performance. Animal. 1(6):872-80

Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: Quality assessment tool for genome assemblies. Bioinformatics. 2013;29:1072–5.

Han Y, Renu S, Patil V, Schrock J, Feliciano-Ruiz N, Selvaraj R, et al. Immune Response to Salmonella Enteritidis Infection in Broilers Immunized Orally With Chitosan-Based Salmonella Subunit Nanoparticle Vaccine. Front Immunol. 2020;11.

Han Z, Sun J, Lv A, Wang A. 2019. Biases from different DNA extraction methods in intestine microbiome research based on 16S rDNA sequencing: a case in the koi carp, Cyprinus carpio var. Koi. Microbiologyopen. 8(1):e00626. doi: 10.1002/mbo3.626.

Harmsen, H.J.M.,Wildeboer–Veloo, A.C.M., Raangs, G.C., Wagendorp, A.A., Klijn, N., Bindels, J.G.; Welling, G.W. 2000. Analysis of Intestinal Flora Development in Breast-

Fed and Formula-Fed Infants by Using Molecular Identification and Detection Methods. J. Ped. Gastroen. Nutr. 30:61-67

Harrison AP, Hansen PA. Bacteroides hypermegas nov. spec. Antonie Van Leeuwenhoek. 1963;29:22–8.

Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature. 2010;464:908–12.

Henry LP, Bruijning M, Forsberg SKG, Ayroles JF. The microbiome extends host evolutionary potential. Nat Commun. 2021;12:5141.

Hird SM. Evolutionary Biology Needs Wild Microbiomes. Front Microbiol. 2017;8.

Holman, D.B., Brunelle, B.W., Trachsel, J. and Allen H.K. 2017. Meta-analysis To Define a Core Microbiota in the Swine Gut. mSystems. 2:e00004-17.

Hong, Y. H., H. S. Lillehoj, E. P. Lillehoj, and S. H. Lee. 2006. Changes in immune-related gene expression and intestinal lymphocyte subpopulations following Eimeria maxima infection of chickens. Vet. Immunol. Immunopathol. 114:259–272. doi:10.1016/j.vetimm.2006.08.006.

Honjo K, Hagiwara T, Itoh K, Takahashi E, Hirota Y. Immunohistochemical Analysis of Tissue Distribution of B and T Cells in Germfree and Conventional Chickens. Journal of Veterinary Medical Science. 1993;55:1031–4.

Hooper L V., Xu J, Falk PG, Midtvedt T, Gordon JI. A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proceedings of the National Academy of Sciences. 1999;96:9833–8.

Hooper, L. V. 2009. Do symbiotic bacteria subvert host immunity? Nat. Rev. Microbiol. 7:367–374.

Hou Q, Kwok LY, Zheng Y, Wang L, Guo Z, Zhang J, Huang W, Wang Y, Leng L, Li H, Zhang H. Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. Sci Rep. 2016 Nov 23;6:37376. doi: 10.1038/srep37376.

Hou, C., Liu, H., Zhang, J., Zhang, S., Yang, F., Zeng, X., Thacke, P.A., Zhang, G.and S. Qiao. 2015. Intestinal microbiota succession and immunomodulatory consequences after introduction of Lactobacillus reuteri I5007 in neonatal piglets. PLoS One. 10:1–17.

Hrncir, T., Stepankova, R., Kozakova, H., Hudcovic, T. and Tlaskalova-Hogenova, H. 2008. Gut microbiota and lipopolysaccharide content of the diet influence development of regulatory T cells: Studies in germ-free mice. BMC Immunol. 9:1–11.

Hu, J., Yu, H., Kulkarni, R. R., Sharif, S., Cui, S.W., Xie M. Y., Nie, S. P., Gong J. 2015 Modulation of cytokine gene expression by selected Lactobacillus isolates in the ileum, caecal tonsils and spleen of Salmonella-challenged broilers, Avian Pathology, 44:6, 463-469, DOI: 10.1080/03079457.2015.1086725

Hume, M. E., D. E. Corrier, S. Ambrus, A. Hinton, and J. R. DeLoach. 1993. Effectiveness of dietary propionic acid in controlling Salmonella typhimurium colonization in broiler chicks. Avian Dis. 37:1051–1056. doi:10.2307/1591912.

Humphreys C. Intestinal Permeability.

Hviid, A., Svanström, H. and Frisch, M. 2011. Antibiotic use and inflammatory bowel diseases in childhood. Gut. 60:49–54.

Iino, T., K. Mori, K. Tanaka, K. I. Suzuki, and S. Harayama. 2007. Oscillibacter valericigenes gen. nov., sp. nov., a valerate-producing anaerobic bacterium isolated from the alimentary canal of a Japanese corbicula clam. Int. J. Syst. Evol. Microbiol. 57:1840–1845. doi:10.1099/ijs.0.64717-0.

Ilina, L.A., Yildirim, E.A., Nikonov, I.N., Filippova, V.A., Laptev, G.Y., Novikova, N.I., Grozina, A.A., Lenkova, T.N., Manukyan, V.A., Egorov, I.A. and Fisinin, V. I. 2016. Metagenomic bacterial community profiles of chicken embryo gastrointestinal tract by using T-RFLP analysis. Dokl. Biochem. Biophys. 466:47–51.

Impey CS, Mead GC, George SM. Competitive exclusion of salmonellas from the chick caecum using a defined mixture of bacterial isolates from the caecal microflora of an adult bird. Journal of Hygiene. 1982;89:479–90.

Ipharraguerre, I., Pastor, J., Gavalda-Navarro, A., Villarroya, F., and Mereu, A. 2018. Anitmicrobial promotion of pig tissue is associated with tissue-specific remodeling bile acids signature and signalling. Sci. Rep. 8(13671):1-13.

Jackson, K.M. and Nazar, A.M. 2006. Breastfeeding , the Immune Response , and Long-term Health. J. Am. Osteopath. Assoc. 106:203–207.

Jacobsen, C.N., Nielsen, V.R., Hayford, A.E., Møller, P.L. Michaelsen, A., Pærregaard, B. Sandström, M. Tvede and Jakobsen M. 1999. Screening of Probiotic Activities of Forty-

Seven Strains of Lactobacillus spp. by In Vitro Techniques and Evaluation of the Colonization Ability of Five Selected Strains in Humans. App. Environ. Microbiol. 65: 4949-4956.

Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018;9.

Janzcyk, P., Pieper, R., Souffrant, W.B., Bimczok, D., Rothkotter, H.J. and Smidt, H. 2007. Parenteral long-acting amoxicillin reduces intestinal bacterial community diversity in piglets even 5 weeks after the administration. ISME Journal 1: 180-183.

Janzen DH. When is it Coevolution? 1980.

Jensen, P. and Recén, B. 1989. When to wean — Observations from free-ranging domestic pigs. App. Anim. Behav. Sci. 23: 49-60.

Jha R, Das R, Oak S, Mishra P. Probiotics (Direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Animals. 2020;10:1–19.

Jiménez, E., Fernández, L., Marín, M.L., Martín, R., Odriozola, J.M., Nueno-Palop, C., Narbad, A., Olivares, M., Xaus, J., and Rodríguez, J.M. 2005. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr. Microbiol. 51:270–274.

Johnson TJ, Youmans BP, Noll S, Cardona C, Evans NP, Peter Karnezos T, et al. A consistent and predictable commercial broiler chicken bacterial microbiota in antibiotic-free production displays strong correlations with performance. Appl Environ Microbiol. 2018;84.

Johnson, A.K., Mcglone, J.J., and Morrow-Tesch, J. L. 2001. Behavior and performance of lactating sows and piglets reared indoors or outdoors. J. Anim. Sci. 79:2571–2579.

Jones EW, Carlson JM, Sivak DA, Ludington WB. Stochastic microbiome assembly depends on context. Proceedings of the National Academy of Sciences. 2022;119.

Jones J, Reinke SN, Ali A, Palmer DJ, Christophersen CT. Fecal sample collection methods and time of day impact microbiome composition and short chain fatty acid concentrations. Sci Rep. 2021;11.

Jones, E. W., Carlson, J. M., Sivak, D. A., Ludington, W. B., 2022. Stochastic microbiome assembly depends on context. PNAS. 119 (7) e2115877119 https://doi.org/10.1073/pnas.2115877119

Joyce, S.A. 2016. Bile acid modification at the host interface:potential for nutraceutical and pharmaceutical interventions in host health. Annu. Rev. Food Science. 7:313-333.

Józefiak D, Rutkowski A, Martin SA. Carbohydrate fermentation in the avian ceca: A review. Animal Feed Science and Technology. 2004;113:1–15.

Ju T, Bourrie BCT, Forgie AJ, Pepin DM, Tollenaar S, Sergi CM, et al. The Gut Commensal Escherichia coli Aggravates High-Fat-Diet-Induced Obesity and Insulin Resistance in Mice. Appl Environ Microbiol. 2023;89.

Ju, T., Kong, J.Y., Stothard, P., Willing, B.P. 2019. Defining the role of Parasutterella, a previously uncharacterized member of the core gut microbiota. ISME Journal. (in press)

Ju, T., Shoblak, Y., Gao, Y., Yang, K., Fouhse, J., So, Y. and Willing, B. 2017. The initial gut microbial composition is a key factor driving host responses to antibiotic treatment. Appl. Env. Microb. 83:e01107-17.

Jurburg SD, Brouwer MSM, Ceccarelli D, van der Goot J, Jansman AJM, Bossers A. Patterns of community assembly in the developing chicken microbiome reveal rapid primary succession. Microbiologyopen. 2019;8.

Kabir SML. The role of probiotics in the poultry industry. International Journal of Molecular Sciences. 2009;10:3531–46.

Kaiser, P., L. Rothwell, E. E. Galyov, P. A. Barrow, J. Burnside, and P. Wigley. 2000. Differential cytokine expression in avian cells in response to invasion by Salmonella typhimurium, Salmonella enteritidis and Salmonella gallinarum. Microbiology. 146:3217–3226. doi:10.1099/00221287-146-12-3217.

Karasova D, Faldynova M, Matiasovicova J, Sebkova A, Crhanova M, Kubasova T, et al. Host Species Adaptation of Obligate Gut Anaerobes Is Dependent on Their Environmental Survival. Microorganisms. 2022;10.

Kassambara A. 2022. ggpubr: 'ggplot2' Based Publication Ready Plots_. R package version 0.5.0, https://CRAN.R-project.org/package=ggpubr

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30:772–780. doi:10.1093/molbev/mst010.

Kayal A, Stanley D, Radovanovic A, Horyanto D, Van TTH, Bajagai YS. Controlled Intestinal Microbiota Colonisation in Broilers under the Industrial Production System. Animals. 2022;12.

Kerr AK, Farrar AM, Waddell LA, Wilkins W, Wilhelm BJ, Bucher O, et al. A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on Salmonella spp. prevalence and concentration in broiler chickens. Prev Vet Med. 2013;111:112–25.

Kers, J.G., Velkers, F.C., Fischer, E.A., Hermes, G.D.A, Stegeman, J.A. and Smidt, H. 2018. Host and environmental factors affecting the intestinal microbiota in chickens. Front. Microbiol. 9:1–14.

Klasing KC, Laãoerin DE, Peng RK, Fry AM. Immunologically Mediated Growth Depression in Chicks: Influence of Feed Intake, Corticosterone and Interleukin-11.

Klasing KC. Nutritional Modulation of Resistance to Infectious Diseases.

Kogut, M. H., and Arsenault, R. J. 2017. Immunometabolic phenotype alterations associated with the induction of disease tolerance and persistent asymptomatic infection of Salmonella in the chicken intestine. Front. Immunol. 8:1–7.

Kogut, M.H.; Swaggerty, C.L.; Byrd, J.A.; Selvaraj, R.; Arsenault, R.J. 2016. Chicken-Specific Kinome Array Reveals that Salmonella enterica Serovar Enteritidis Modulates Host Immune Signaling Pathways in the Cecum to Establish a Persistence Infection. Int. J. Mol. Sci. 2016, 17, 1207. https://doi.org/10.3390/ijms17081207

Kohl, K.D., Skopec, M.M. and Dearing, M.D. 2014. Captivity results in disparate loss of gut microbial diversity in closely related hosts. Conserv. Physiol. 2:1–11.

Kolde, R., 2019. pheatmap: Pretty Heatmaps. R package version 1.0.12, https://CRAN.R-project.org/package=pheatmap

Kollarcikova M, Faldynova M, Matiasovicova J, Jahodarova E, Kubasova T, Seidlerova Z, Babak V, Videnska P, Cizek A, Rychlik I. 2020. Different Bacteroides Species Colonise Human and Chicken Intestinal Tract. Microorganisms. 8(10):1483. doi: 10.3390/microorganisms8101483.

Konstantinov, S. R., Awati, A. A., Williams, B. A., Miller, B. G., Jones, P., Stokes, C. R., Akkermans, A. D. L., Smidt, H. and Vos, W.M. 2006. Post-natal development of the porcine microbiota composition and activities. Environ. Microbiol. 8: 1191-1199.

Korver, D. R. 2006. Overview of the Immune Dynamics of the Digestive System. J. App. Poultry Res. 15: 123-135.

Kraehenbuhl, J.P., Pringault, E., and Neutra, M.R. 2010. Review article: Intestinal epithelia and barrier functions. Aliment. Pharmacol. Ther. 11:3–9

Krysiak K, Konkol D, Korczyński M. Overview of the Use of Probiotics in Poultry Production. Animals. 2021;11:1620.

Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, et al. 2019. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS ONE 14(3): e0212446. https://doi.org/10.1371/journal.pone.0212446

Kubasova T, Seidlerova Z, Rychlik I. Ecological adaptations of gut microbiota members and their consequences for use as a new generation of probiotics. International Journal of Molecular Sciences. 2021;22.

Kubasova, T., M. Kollarcikova, M. Crhanova, D. Karasova, D. Cejkova, A. Sebkova, J. Matiasovicova, M. Faldynova, F. Sisak, V. Babak, A. Pokorna, A. Cizek, and I. Rychlik. 2019. Gut anaerobes capable of chicken caecum colonisation. Microorganisms. 7:1–11. doi:10.3390/microorganisms7120597.

Kuhn, K. A., H. M. Schulz, E. H. Regner, E. L. Severs, J. D. Hendrickson, G. Mehta, A. K. Whitney, D. Ir, N. Ohri, C. E. Robertson, D. N. Frank, E. L. Campbell, and S. P. Colgan. 2018. Bacteroidales recruit IL-6-producing intraepithelial lymphocytes in the colon to promote barrier integrity. Mucosal Immunol. 11:357–368. doi:10.1038/mi.2017.55.

Lagier JC, Dubourg G, Million M, Cadoret F, Bilen M, Fenollar F, et al. Culturing the human microbiota and culturomics. Nature Reviews Microbiology. 2018;16:540–50.

Lahti, L., and S. Shetty. 2018. Introduction to the microbiome R package. Bioconductor. 1–71.

Langella P, Guarner F, Martín R. Editorial: Next-generation probiotics: From commensal bacteria to novel drugs and food supplements. Frontiers in Microbiology. 2019;10 AUG.

Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013;31:814–21.

Law, C. W., Y. Chen, W. Shi, and G. K. Smyth. 2014. Voom: Precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol. 15:1–17. doi:10.1186/gb-2014-15-2-r29.

Lawal RA, Hanotte O. Domestic chicken diversity: Origin, distribution, and adaptation. Animal Genetics. 2021;52:385–94.

Lazar V, Ditu L-M, Pircalabioru GG, Gheorghe I, Curutiu C, Holban AM, et al. Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer. Front Immunol. 2018;9.

Lee KC, Kil DY, Sul WJ. Cecal microbiome divergence of broiler chickens by sex and body weight. Journal of Microbiology. 2017;55:939–45.

Lee MD, Ipharraguerre IR, Arsenault RJ, Lyte M, Lyte JM, Humphrey B, et al. Informal nutrition symposium: leveraging the microbiome (and the metabolome) for poultry production. Poult Sci. 2022;101:101588.

Lee, I.K., Gu, M.J., Ko, K.H., Bae, S., Kim, G., Jin, G.D, Kim, E.B, Kong, Y.Y., Park, T.S., Park, B.C., Jung, H.J., Han, S.H., and Yun, C.H. 2018. Regulation of CD4+CD8-CD25+ and CD4+CD8+CD25+ T cells by gut microbiota in chicken. Sci. Rep. 8:1–11.

Leitner G, Dan Heller E, Friedman A. Sex-related differences in immune response and survival rate of broiler chickens. Vet Immunol Immunopathol. 1989;21:249–60.

Leong LEX, Khan S, Davis CK, Denman SE, McSweeney CS. Fluoroacetate in plants - a review of its distribution, toxicity to livestock and microbial detoxification. Journal of Animal Science and Biotechnology. 2017;8.

Lev M, Forbes M. Growth response to dietary penicillin of germ-free chicks and of chicks with a defined intestinal flora. British Journal of Nutrition. 1959;13:78–84.

Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006;124:837–48.

Ley, R., Turnbaugh, P., Klein, S., Gordon, J. 2006. Human gut microbes associated with obesity. Nature 444:1022-1023. https://doi.org/10.1038/4441022a

Ley, R.E., Hamady, M., Lozupone, C., Turnbaugh, P.J., Ramey, R.R., Bircher, J.S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., and Gordon, J.I. 2008. Evolution of mammals and Their Gut Microbes. Science. 320:1647–1652.

Li, G., Yao, W. and Jiang H. 2014. Short-chain fatty acids enhance adipocytes differentiation in the stromal of vascular fraction of porcine tissue. J. Nut. 144: 1887-95

Li, J., Yang, K., Ju, T., Ho, T., McKay, C.A., Gao, Y., Forget, S.K., Gartner, S.R., Field C.J., Chan, C.B. and Willing, B.P., 2017. Early life antibiotic exposure affects pancreatic islet development and metabolic regulation. Sci. Rep. 7 (41778):1-12.

Li, P., M. Gao, B. Song, Y. Liu, S. Yan, J. Lei, Y. Zhao, G. Li, T. Mahmood, Z. Lv, Y. Hu, and Y. Guo. 2022. Fecal microbiota transplantation reshapes the physiological function of the intestine in antibiotic-treated specific pathogen-free birds. Front. Immunol. 13:1–13. doi:10.3389/fimmu.2022.884615.

Lin J. Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. Frontiers in Microbiology. 2014;5 FEB.

Lin, H., and S. Das Peddada. 2020. Analysis of compositions of microbiomes with bias correction. Nat. Commun. 11:1–11. doi:10.1038/s41467-020-17041-7.

Litvak Y, Mon KKZ, Nguyen H, Chanthavixay G, Liou M, Velazquez EM, et al. Commensal Enterobacteriaceae Protect against Salmonella Colonization through Oxygen Competition. Cell Host Microbe. 2019;25:128-139.e5.

Liu L, Li Q, Yang Y, Guo A. Biological Function of Short-Chain Fatty Acids and Its Regulation on Intestinal Health of Poultry. Front Vet Sci. 2021;8.

Liu, Z., L. Wang, Y. Yu, A. Fotin, Q. Wang, P. Gao, Y. Zhang, T. Fotina, and J. Ma. 2022. SteE Enhances the virulence of Salmonella Pullorum in chickens by regulating the inflammation response. Front. Vet. Sci. 9:1–12. doi:10.3389/fvets.2022.926505.

López-García A, Pineda-Quiroga C, Atxaerandio R, Pérez A, Hernández I, García-Rodríguez A and González-Recio O. 2018. Comparison of Mothur and QIIME for the Analysis of Rumen Microbiota Composition Based on 16S rRNA Amplicon Sequences. Front. Microbiol. 9:3010. doi: 10.3389/fmicb.2018.03010

Lopez-Jaramillo P., Arbeláez D.G., López J.L., López, C., Martínez-Ortega, J. Rodríguez, A. G., Cubillos, S. T. 2014. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. Horm Mol Biol Clin Investig. 18(1):37-45

Louca S, Doebeli M. Efficient comparative phylogenetics on large trees. Bioinformatics. 2018;34:1053–5.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:1–21. doi:10.1186/s13059-014-0550-8.

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K. and Knight, R. 2012. Diversity, stability, and resilience of the human gut microbiota. Nature. 489(7415):220-230.

Lu J, Idris U, Harmon B, Hofacre C, Maurer JJ, Lee MD. Diversity and Succession of the Intestinal Bacterial Community of the Maturing Broiler Chicken. Appl Environ Microbiol. 2003;69:6816–24.

Lundberg R, Scharch C, Sandvang D. The link between broiler flock heterogeneity and cecal microbiome composition. Anim Microbiome. 2021;3.

Luo, J., C. S. Ranadheera, S. King, C. A. Evans, and S. Baines. 2018. Dairy propionibacteria as direct-fed microbials: in vitro effect on acid metabolism of Streptococcus bovis and Megasphaera elsdenii. Ann. Microbiol. 68:153–158. doi:10.1007/s13213-018-1322-6.

Lupp, C., Robertson, M.L., Wickham, M.E., Sekirov, I., Champion, O.L., Gaynor, E.C. and Finlay, B.B. 2007. Host-Mediated Inflammation Disrupts the Intestinal Microbiota and Promotes the Overgrowth of Enterobacteriaceae. Cell Host Microbe. 2:119–129.

Lynagh, G. R., M. Bailey, and P. Kaiser. 2000. Interleukin-6 is produced during both murine and avian Eimeria infections. Vet. Immunol. Immunopathol. 76:89–102. doi:10.1016/S0165-2427(00)00203-8.

Ma H, Liu H, Zhang L, Yang M, Fu B, Liu H. Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. Biotechnol Biofuels. 2017;10:137.

Maki JJ, Looft T. 2018. Megasphaera stantonii sp. nov., a butyrate-producing bacterium isolated from the cecum of a healthy chicken. Int J Syst Evol Microbiol. 68(11):3409-3415. doi: 10.1099/ijsem.0.002991.

Maldonado-Gómez, M.X., Martínez, I., Bottacini, F., O'Callaghan, A., Ventura, M., van Sinderen, D., Hillmann, B., Vangay, P., Knights, D., Hutkins, R. W. and Walter, J. 2016. Stable Engraftment of Bifidobacterium longum AH1206 in the Human Gut Depends on Individualized Features of the Resident Microbiome. Cell Host Microbe. 20:515–526.

Mancabelli L, Ferrario C, Milani C, Mangifesta M, Turroni F, Duranti S, et al. Insights into the biodiversity of the gut microbiota of broiler chickens. Environ Microbiol. 2016;18:4727–38.

Marais, M., S. K. Maloney, and D. A. Gray. 2011. Brain IL-6- and PG-dependent actions of IL-1β and lipopolysaccharide in avian fever. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 301. doi:10.1152/ajpregu.00136.2011.

Marcolla C, Ju T, Lantz HL, Willing BP. Investigating the cecal microbiota of broilers raised in extensive and intensive production systems. Res Sq. 2023.

Marcolla CS, Ju T, Willing BP. Cecal Microbiota Development and Physiological Responses of Broilers Following Early Life Microbial Inoculation Using Different Delivery Methods and Microbial Sources. Appl Environ Microbiol. 2023;89.

Marcolla, C.S., Alvarado, C.S., and Willing, B. P. 2019. Early life microbial exposure shapes subsequent animal health. Canadian Journal of Animal Science. 99(4): 661-677. https://doi.org/10.1139/cjas-2019-0029

Martin, C., Ling, P. and Blackburn, G. 2016. Review of infant feeding: Key features of breast milk and infant formula. Nutrients 8: 279.

Martínez I, Stegen JC, Maldonado-Gómez MX, Eren MA, Siba PM, Greenhill AR, et al. The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes. Cell Rep. 2015;11:527–38.

Matamoros, S., Gras-Leguen, C., Le Vacon, F., Potel, G., and De La Cochetiere, M.F. 2013. Development of intestinal microbiota in infants and its impact on health. Trends Microbiol. 21:167–173.

Mazmanian, S. K., Liu, C.H., Tzianabos, A.O. and Kasper, D.L. 2005. An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System. Cell 122: 107-118.

McBride, G., I. P. Parer, and F. Foenander. 1969. The social organization and behaviour of the feral domestic fowl. Anim. Behav. Monogr. 2:125–181. doi:10.1016/s0066-1856(69)80003-8.

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 8. doi:10.1371/journal.pone.0061217.

Mead GC. Microbes of the Avian Cecum: Types Present and Substrates Utilized. Journal of Experimental Zoology.1989;252(S3):48-54.

Medvecky M, Cejkova D, Polansky O, Karasova D, Kubasova T, Cizek A, et al. Whole genome sequencing and function prediction of 133 gut anaerobes isolated from chicken caecum in pure cultures. BMC Genomics. 2018;19.

Meijerink, N., J. G. Kers, F. C. Velkers, D. A. van Haarlem, D. M. Lamot, J. E. de Oliveira, H. Smidt, J. A. Stegeman, V. P. M. G. Rutten, and C. A. Jansen. 2020. Early life inoculation with adult-derived microbiota accelerates maturation of intestinal microbiota and enhances NK cell activation in broiler chickens. Front. Vet. Sci. 7. doi:10.3389/fvets.2020.584561.

Menendez, A., Willing, B.P., Montero, M., Wlodarska, M., x So, M., Bhinder, G., Vallance, B. A. and Finlay, B.B. 2013. Bacterial stimulation of the TLR-MyD88 pathway modulates the homeostatic expression of ileal paneth cell α-defensins. J. Innate Immun. 5:39–49.

Michael, Waturangi DE. Antibiofilm activity from endophyte bacteria, Vibrio cholerae strains, and actinomycetes isolates in liquid and solid culture. BMC Microbiol. 2023;23:83.

Microsoft Corporation. 2018. Microsoft Excel

Miller, B., Newby, T.J., Stokes, C.R., Hampson, D. and Bourne, F.J. 1983Ann Rech Vet, 14:487-492.

Miquel, S., Martin, R., Bridonneau, C., Robert, V., Sokol, H., Bermúdez-Humarán, L. G., Thomas, M. and Langella, P. 2014. Ecology and metabolism of the beneficial intestinal commensal bacterium Faecalibacterium prausnitzii. Gut Microbes. 5:146–151.

Moore PR, Evenson A, Luckey TD, Mccoy E, Elvehjem CA, Hart EB. Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick*.

Moote PE, Zaytsoff SJM, Ortega Polo R, Abbott DW, Uwiera RRE, Douglas Inglis G. Application of culturomics to characterize diverse anaerobic bacteria from the gastrointestinal tract of broiler chickens in relation to environmental reservoirs. Can J Microbiol. 2020;66:288–302.

Mormède, P. and Hay, M. Behavioural changes and adaptation associated with weaning. In: Pluske, J.R., Le Dividich, J., Verstegen, M.W.A. (ed.). Weaning the pig: concepts and consequences. Netherlands:Wagening Academic Publishers, 2003. chap.4. p. 51-59.

Moya A, Ferrer M. Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance. Trends in Microbiology. 2016;24:402–13.

Mulder, I.E., Schmidt, B., Stokes, C.R., Lewis, M., Bailey, M., Aminov, R.I., Prosser, J.I., Gill, B.P., Pluske, J.R., Mayer, C.D., Musk C.C, and Kelly, D. 2009. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biology 7: 79.

Muramatsu T, Takasu O, Furuse M, Tasaki I, Okumura J. Influence of the gut microflora on protein synthesis in tissues and in the whole body of chicks. Biochemical Journal. 1987;246:475–9.

Nakamura A, Ota Y, Mizukami A, Ito T, Ngwai YB, Adachi Y. Evaluation of Aviguard, a Commercial Competitive Exclusion Product for Efficacy and After-Effect on the Antibody Response of Chicks to Salmonella.

Nan S, Yao M, Zhang X, Wang H, Li J, Niu J, et al. Fermented grape seed meal promotes broiler growth and reduces abdominal fat deposition through intestinal microorganisms. Front Microbiol. 2022;13.

Nearing, J.T., Douglas, G.M., Hayes, M.G. et al. 2022. Microbiome differential abundance methods produce different results across 38 datasets. Nat Commun 13, 342. https://doi.org/10.1038/s41467-022-28034-

Neu, A. T. 2021. Defining and quantifying the core microbiome : Challenges and prospects. 118:1–10. doi:10.1073/pnas.2104429118/-/DCSupplemental.Published.

Newburg, D.S. 2000.Oligosaccharides in human milk and bacterial colonization. J Pediatr Gastroenterol Nutr, 30:S8-S17.

Ngunjiri JM, Taylor KJM, Abundo MC, Jang H, Elaish M, Mahesh KC, et al. Farm stage, bird age, and body site dominantly affect the quantity, taxonomic composition, and dynamics of respiratory and gut microbiota of commercial layer chickens. Appl Environ Microbiol. 2019;85.

Nisbet, D. J., D. E. Corrier, S. C. Ricke, M. E. Hume, J. A. Byrd, and J. R. DeLoach. 1996. Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to Salmonella in chicks. Anaerobe. 2:345–350. doi:10.1006/anae.1996.0044.

Nishimichi, N., T. Kawashima, S. Hojyo, H. Horiuchi, S. Furusawa, and H. Matsuda. 2006. Characterization and expression analysis of a chicken interleukin-6 receptor alpha. Dev. Comp. Immunol. 30:419–429. doi:10.1016/j.dci.2005.05.007.

Notterpek, L., Roux, K.J., Amici, S.A., Yazdanpour, A., Rahner, C. and Fletcher. B.S., 2001. Peripheral myelin protein 22 is a constituent of intercellular junctions in epithelia. Proc. Natl. Acad. Sci. 98:14404–14409.

Nurmi E, Nuotio L, Schneitz C. The competitive exclusion concept: development and future. Int J Food Microbiol. 1992;15:237–40.

Nurmi, E., and Rantala, M. 1973. New aspects of Salmonella infection in Broiler production. Nature 241:210-211. doi: https://doi.org/10.1038/241210a0

Ocejo, M., Oporto, B. & Hurtado, A. 2019. 16S rRNA amplicon sequencing characterization of caecal microbiome composition of broilers and free-range slow-growing chickens throughout their productive lifespan. Sci Rep 9, 2506 https://doi.org/10.1038/s41598-019-39323-x

Odle, J., Zijlstra, R.T. and Donovan, S.M. 1996. Intestinal Effects of Milkborne Growth Factors in Neonates of Agricultural Importance. J. Anim. Sci. 74:2509–2522.

OECD. 2022.

Oh PL, Benson AK, Peterson DA, Patil PB, Moriyama EN, Roos S, et al. Diversification of the gut symbiont lactobacillus reuteri as a result of host-driven evolution. ISME Journal. 2010;4:377–87.

Oh S, Lillehoj HS, Lee Y, Bravo D, Lillehoj EP. Dietary Antibiotic Growth Promoters Down-Regulate Intestinal Inflammatory Cytokine Expression in Chickens Challenged With LPS or Co-infected With Eimeria maxima and Clostridium perfringens. Front Vet Sci. 2019;6.

Oksanen J., Simpson G., Blanchet F., Kindt R., Legendre P., Minchin P., O'Hara R., Solymos P., Stevens M., Szoecs E., Wagner H., Barbour M., Bedward M., Bolker B., Borcard D., Carvalho G., Chirico M., De Caceres M., Durand S., Evangelista H., FitzJohn R., Friendly M., Furneaux B, Hannigan G., Hill M., Lahti L., McGlinn D., Ouellette M., Ribeiro Cunha E., Smith T., Stier A., Ter Braak C., Weedon J. 2020. vegan: Community Ecology Package. R package version 2.5-7., https://CRAN.R-project.org/package=vegan.

Olesiuk, O. M., G. H. Snoeyenbos, and C. F. Smyser. 1971. Inhibitory effect of used litter on Salmonella typhimurium transmission in the chicken. Avian Dis. 15:118–124. doi:10.2307/1588396.

Onrust, L., K. Van Driessche, R. Ducatelle, K. Schwarzer, F. Haesebrouck, and F. Van Immerseel. 2018. Valeric acid glyceride esters in feed promote broiler performance and reduce the incidence of necrotic enteritis. Poult. Sci. 97:2303–2311. doi:10.3382/ps/pey085.

Ott, S.J., Musfeldt, M., Wenderoth, D.F., Hampe, J., Brant, O., Fölsch, U.R., Timmis, K.N. and Schreiber, S. 2004. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut. 53:685–693.

Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014;42.

Pan, D., and Z. Yu. 2014. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 5:108–119. doi:10.4161/gmic.26945.

Pandit RJ, Hinsu AT, Patel N V., Koringa PG, Jakhesara SJ, Thakkar JR, et al. Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16s rDNA amplicon sequencing. Microbiome. 2018;6.

Park, S. S., Lillehoj, H. S., Allen, P. C., Park, D. W., FitzCoy, S., Bautista, D. A., & Lilleho, E. P. 2008. Immunopathology and Cytokine Responses in Broiler Chickens Coinfected with Eimeria Maxima and Clostridium perfringens with the Use of an Animal Model of Necrotic Enteritis. Avian Diseases, 52(1), 14–22. http://www.jstor.org/stable/27565692

Parry, S. H., W. D. Allen, and P. Porter. 1977. Intestinal immune response to E. coli antigens in the germ-free chicken. Immunology. 32:731–41.

Paul SS, Chatterjee RN, Raju MVLN, Prakash B, Rao SVR, Yadav SP, et al. Gut microbial composition differs extensively among indian native chicken breeds originated in different geographical locations and a commercial broiler line, but breed-specific, as well as across-breed core microbiomes, are found. Microorganisms. 2021;9:1–22.

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D.,

Brucher, M., Perrot, M., Duchesnay, E. 2011. Scikit-learn: machine Learning in Python. Journal of Machine Learning Research. 12:2825-2830.

Pedroso AA, Lee MD, Maurer JJ. Strength Lies in Diversity: How Community Diversity Limits Salmonella Abundance in the Chicken Intestine. Front Microbiol. 2021;12.

Pedroso, A.A. 2009. Which came first: the egg or its microbiota? Poult. Inf. Prof. 1–5.

Perez-Muñoz, M.E., Arrieta, M.C., Ramer-Tait, A.E. and Walter, J. 2017. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: Implications for research on the pioneer infant microbiome. Microbiome. 5:1–19.

Pham OH, McSorley SJ. Protective host immune responses to Salmonella infection. Future Microbiol. 2015;10:101–10.

Pi, Y., Gao, K., Peng, Y., Mu, C.L., Zhu, W.Y. 2019. Antibiotic-induced alterations of the gut microbiota and microbial fermentation in protein parallel the changes in host nitrogen metabolism of growing pigs. Animal. 13(2):262-272

Pluske, J.R., Hampson, D.J., and Willians, I.H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci., 51:215-236.

Polansky, O., Z. Sekelova, M. Faldynova, A. Sebkova, F. Sisak, I. Rychlik, L. 2016. Important metabolic pathways and biological processes expressed by chicken cecal Microbiota. 82:1569–1576. doi: 10.1128/AEM.03473-15

Pottenger S, Watts A, Wedley A, Jopson S, Darby AC, Wigley P. Timing and delivery route effects of cecal microbiome transplants on Salmonella Typhimurium infections in chickens: potential for in-hatchery delivery of microbial interventions. Anim Microbiome. 2023;5.

Poudel, B., N. Shterzer, Y. Sbehat, N. Ben-Porat, M. Rakover, R. Tovy-Sharon, D. Wolicki, S. Rahamim, E. Bar-Shira, and E. Mills. 2022. Characterizing the chicken gut colonization ability of a diverse group of bacteria. Poult. Sci. 101:102136. doi:10.1016/j.psj.2022.102136.

Prakasam, R., M. Fujimoto, R. Takii, N. Hayashida, E. Takaki, K. Tan, F. Wu, S. Inouye, and A. Nakai. 2013. Chicken IL-6 is a heat-shock gene. FEBS Lett. 587:3541–3547. doi:10.1016/j.febslet.2013.09.012.

Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS One. 5. doi:10.1371/journal.pone.0009490.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41:590–596. doi:10.1093/nar/gks1219.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S. and Medzhitov, R. 2004. Recognition of Commensal Microflora by Toll-Like Receptors Is Required for Intestinal Homeostasis. Cell 118: 229-241.

Ramírez, G. A., E. Richardson, J. Clark, J. Keshri, Y. Drechsler, M. E. Berrang, R. J. Meinersmann, N. A. Cox, and B. B. Oakley. 2020. Broiler chickens and early life programming: Microbiome transplant-induced cecal community dynamics and phenotypic effects. PLoS One. 15:1–22. doi:10.1371/journal.pone.0242108.

Rantala, M., and E. Nurmi. 1973. Prevention of the growth of Salmonella infantis in chicks by the flora of the alimentary tract of chickens. Br. Poult. Sci. 14:627–630. doi:10.1080/00071667308416073.

Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal Gut Microbiota Transplants from Zebrafish and Mice to Germ-free Recipients Reveal Host Habitat Selection. Cell. 2006;127:423–33.

Redweik GAJ, Millman ST, Parsons RL, Hurtado Terminel AN, Radkowski R, Daniels K, et al. Exposure to a Virtual Environment Induces Biological and Microbiota Changes in Onset-of-Lay Hens. Front Virtual Real. 2022;3.

Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, et al. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. ISME Journal. 2014;8:1323–35.

Revelle W. psych: Procedures for Psychological, Psychometric, and Personality Research. 2023.

Revolledo L, Ferreira CSA, Ferreira AJP. Prevention of Salmonella Typhimurium colonization and organ invasion by combination treatment in broiler chicks. Poult Sci. 2009;88:734–43.

Ringseis R, Eder K. Heat stress in pigs and broilers: role of gut dysbiosis in the impairment of the gut-liver axis and restoration of these effects by probiotics, prebiotics and synbiotics. J Anim Sci Biotechnol. 2022;13:126.

Ríos-Covian D, Langella P, Martín R. From Short- to Long-Term Effects of C-Section Delivery on Microbiome Establishment and Host Health. Microorganisms. 2021;9:2122.

Ritchie, M. E., B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, and G. K. Smyth. 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 43:e47. doi:10.1093/nar/gkv007.

Robinson K, Assumpcao ALF V., Arsi K, Erf GF, Donoghue A, Jesudhasan PRR. Effect of Salmonella Typhimurium Colonization on Microbiota Maturation and Blood Leukocyte Populations in Broiler Chickens. Animals. 2022;12:2867.

Rose-John, S., K. Winthrop, and L. Calabrese. 2017. The role of IL-6 in host defence against infections: Immunobiology and clinical implications. Nat. Rev. Rheumatol. 13:399–409. doi:10.1038/nrrheum.2017.83.

Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, McCulloch JA, Anastasakis DG, Sarshad AA, Leonardi I, Collins N, Blatter JA, Han SJ, Tamoutounour S, Potapova S, Foster St Claire MB, Yuan W, Sen SK, Dreier MS, Hild B, Hafner M, Wang D, Iliev ID, Belkaid Y, Trinchieri G, Rehermann B. 2019. Laboratory mice born to wild mice have natural microbiota and model human immune responses. Science. 365(6452):eaaw4361. doi: 10.1126/science.aaw4361.

Rosshart, S.P., Vassallo, B.G., Angeletti, D., Hutchinson, D.S., Morgan, A.P., Takeda, K., Hickman, H.D., McCulloch, J.A., Badger, J.H., Ajami, N. J., Trinchieri, G., Pardo-Manuel de Villena, F., Yewdell, J. W., and Rehermann, B. 2017. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. Cell. 171:1015–1028.e13.

Rothwell, L., J. R. Young, R. Zoorob, C. A. Whittaker, P. Hesketh, A. Archer, A. L. Smith, and P. Kaiser. 2004. Cloning and characterization of chicken IL-10 and its role in the immune response to Eimeria maxima. J. Immunol. 173:2675–2682. doi:10.4049/jimmunol.173.4.2675.

Rougière N, Carré B. Comparison of gastrointestinal transit times between chickens from D + and D- genetic lines selected for divergent digestion efficiency. Animal. 2010;4:1861–72.

Round, J. L., and Mazmanian, S.K. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. 9:313–323.

Roura E, Homedes J, Klasing KC. Prevention of Immunologic Stress Contributes to the Growth-Permitting Ability of Dietary Antibiotics in Chicks ,. J Nutr. 1992;122:2383–90.

Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., Tuohy, K., 2018. Gut microbiota functions: metabolism and other food components. Eur J Nutr. 57:1-24.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Rubio LA. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poult Sci. 2019;98:695–706.

Rychlik I. Composition and function of chicken gut microbiota. Animals. 2020;10.

Saint-Martin V, Quéré P, Trapp S, Guabiraba R. Uncovering the core principles of the gut-lung axis to enhance innate immunity in the chicken. 2022. https://doi.org/10.3389/fimmu.2022.956670ï.

Sakon H, Nagai F, Morotomi M, Tanaka R. Sutterella parvirubra sp. nov. and Megamonas funiformis sp. nov., isolated from human faeces. Int J Syst Evol Microbiol. 2008;58:970–5.

Samad, F., G. Bergtrom, H. Eissa, and D. L. Amrani. 1993. Stimulation of chick hepatocyte fibronectin production by fibroblast-conditioned medium is due to interleukin 6. BBA - Mol. Basis Dis. 1181:207–213. doi:10.1016/0925-4439(93)90022-S.

Sansonetti, P. J. 2004. War and peace at mucosal surfaces. Nat. Rev. Imm. 4: 953-964.

Sapan, H. B., I. Paturusi, I. Jusuf, I. Patellongi, M. N. Massi, A. D. Pusponegoro, S. K. Arief, I. Labeda, A. A. Islam, L. Rendy, and M. Hatta. 2016. Pattern of cytokine (IL-6 and IL-10) level as inflammation and anti-inflammation mediator of multiple organ dysfunction syndrome (MODS) in polytrauma. Int. J. Burns Trauma. 6:37–43.

Savage, D. C. 1977. Microbial Ecology of the Gastrointestinal Tract. Annu. Rev. Microbiol. 31:107–133.

Schachtschneider, K.M., Yeoman, C.J., Isaacson, R.E., White, B.A., Schook, L.B. and Pieters, M. 2013. Modulation of Systemic Immune Responses through Commensal Gastrointestinal Microbiota. PLoS One. 8.

Schamberger, G. P., and Diez-Gonzalez. F., 2002. Selection of recently isolated colicinogenic Escherichia coli strains inhibitory to Escherichia coli O157:H7. J. Food Prot. 65:1381–1387.

Schneider, K., R. Klaas, B. Kaspers, and P. Staeheli. 2001. Chicken interleukin-6: cDNA structure and biological properties. Eur. J. Biochem. 268:4200–4206. doi:10.1046/j.1432-1327.2001.02334.x.

Schneitz, C. 2005. Competitive exclusion in poultry - 30 years of research. Food Control. 16:657-667. https://doi.org/10.1016/j.foodcont.2004.06.002.

Schokker, D., Jansman, A.J.M., Veninga, G., de Bruin, N., Vastenhouw, S.A., de Bree, F.M., Bossers, A., Rebel, J.M.J. and Smits. M.A. 2017. Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. BMC Genomics. 18:1–14.

Schokker, D., Zhang, J., Zhang, L.L., Vastenhouw, S.A., Heilig, H.G.H.J., Smidt, H., Rebel, J. M.J., and Smits M.A. 2014. Early-life environmental variation affects intestinal microbiota and immune development in new-born piglets. PLoS One. 9. doi:10.1371/journal.pone.0100040.

Scupham AJ, Jones JA, Rettedal EA, Weber TE. 2010. Antibiotic manipulation of intestinal microbiota to identify microbes associated with Campylobacter jejuni exclusion in poultry. Appl Environ Microbiol. 76(24):8026-32. doi: 10.1128/AEM.00678-10

Scupham, A. J., Patton, T. G., Bent, E. and Bayles, D. O. 2008. Comparison of the cecal microbiota of domestic and wild turkeys. Microb. Ecol. 56:322–331.

Sebők C, Walmsley S, Tráj P, Mackei M, Vörösházi J, Petrilla J, et al. Immunomodulatory effects of chicken cathelicidin-2 on a primary hepatic cell co-culture model. PLoS One. 2022;17 10 October.

Seed, P.C. 2015. The human mycobiome. Cold Spring Harb. Perspect. in Med. 5: a019810.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12.

Seidlerova Z, Kubasova T, Faldynova M, Crhanova M, Karasova D, Babak V, Rychlik I. 2020. Environmental Impact on Differential Composition of Gut Microbiota in Indoor Chickens in Commercial Production and Outdoor, Backyard Chickens. Microorganisms. 8(5):767. doi: 10.3390/microorganisms8050767

Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW, Pallen MJ. Extensive microbial and functional diversity within the chicken cecal microbiome. PLoS One. 2014;9.

Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, et al. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology. 2012;3 DEC.

Shanmugasundaram R, Lourenco J, Hakeem W Al, Dycus MM, Applegate TJ. Subclinical doses of dietary fumonisins and deoxynivalenol cause cecal microbiota dysbiosis in broiler chickens challenged with Clostridium perfringens. Front Microbiol. 2023;14.

Shetty SA, Marathe NP, Lanjekar V, Ranade D, Shouche YS. 2013. Comparative genome analysis of Megasphaera sp. reveals niche specialization and its potential role in the human gut. PLoS One. 2013 Nov 18;8(11):e79353. doi: 10.1371/journal.pone.0079353

Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends in Biotechnology. 2015;33:496–503.

Shin, R., Suzuki, M., and Morishita, Y. 2002. Influence of intestinal anaerobes and organic acids on the growth of enterohaemorrhagic Escherichia coli O157:H7. J. Med. Microbiol. 51:201-206.

Siegerstetter SC, Schmitz-Esser S, Magowan E, Wetzels SU, Zebeli Q, Lawlor PG, et al. Intestinal microbiota profiles associated with low and high residual feed intake in chickens across two geographical locations. PLoS One. 2017;12.

Śliżewska K, Markowiak-Kopeć P, Żbikowski A, Szeleszczuk P. The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. Sci Rep. 2020;10.

Smith, K., McCoy, K.D. and Macpherson, A.J. 2007. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin. Immunol. 19:59–69.

Song B, Li P, Xu H, Wang Z, Yuan J, Zhang B, et al. Effects of rearing system and antibiotic treatment on immune function, gut microbiota and metabolites of broiler chickens. J Anim Sci Biotechnol. 2022;13.

Sonnenburg JL, Xu J, Leip DD, Chen C-H, Westover BP, Weatherford J, et al. Glycan Foraging in Vivo by an Intestine-Adapted Bacterial Symbiont. Science (1979). 2005;307:1955–9.

Souza MR, Moreira JL, Barbosa FH, Cerqueira MM, Nunes AC, Nicoli JR. 2007. Influence of intensive and extensive breeding on lactic acid bacteria isolated from Gallus gallus domesticus ceca. Vet Microbiol. 120(1-2):142-50. doi: 10.1016/j.vetmic.2006.10.019.

Souza, D.G., Vieira, A.T., Soares, A.C., Pinho, V., Nicoli, J.R., Vieira, L.Q., and Teixeira, M.M. 2004. The Essential Role of the Intestinal Microbiota in Facilitating Acute Inflammatory Responses. J. Immunol. 173:4137–4146.

Stanley D, Geier MS, Hughes RJ, Denman SE, Moore RJ. 2013. Highly Variable Microbiota Development in the Chicken Gastrointestinal Tract. PLoS ONE 8(12): e84290. https://doi.org/10.1371/journal.pone.0084290

Stanley, D, Hughes, R.J., Geier, M.S. and Moore R.J. 2016. Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: Challenges presented for the identification of performance enhancing probiotic bacteria. Front. Microbiol. 7:1–13.

Stavric S, Gleeson TM, Blanchfield B, Pivnick H. Competitive Exclusion of Salmonella from Newly Hatched Chicks by Mixtures of Pure Bacterial Cultures Isolated from Fecal and Cecal Contents of Adult Birds. J Food Prot. 1985;48:778–83.

Stecher B, Chaffron S, Käppeli R, Hapfelmeier S, Freedrich S, Weber TC, et al. Like will to like: Abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathog. 2010;6.

Stern NJ, Cox NA, Bailey JS, Berrang ME, Musgrove MT. Comparison of Mucosal Competitive Exclusion and Competitive Exclusion Treatment to Reduce Salmonella and Campylobacter spp. Colonization in Broiler Chickens.

Sueyoshi M, Adachi Y. Diarrhea Induced by Treponema hyodysenteriae: a Young Chick Cecal Model for Swine Dysentery. Infection and Immunity 1990;58(10):3348-3362.

Sun M, Wu W, Chen L, Yang W, Huang X, Ma C, et al. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. Nat Commun. 2018;9.

Sun Y, O'Riordan MXD. Regulation of bacterial pathogenesis by intestinal short-chain fatty acids. In: Advances in Applied Microbiology. Academic Press Inc.; 2013. p. 93–118.

Sun, J., Wang, Y., Li, N., Zhong, H., Xu, H., Zhu, Q., and Liu Y. 2018. Comparative Analysis of the Gut Microbial Composition and Meat Flavor of Two Chicken Breeds in Different Rearing Patterns. Biomed Res. Int. 2018:4343196. doi:10.1155/2018/4343196.

Svihus B, Itani K. Intestinal Passage and Its Relation to Digestive Processes. Journal of Applied Poultry Research. 2019;28:546–55.

Szott V, Reichelt B, Friese A, Roesler U. A Complex Competitive Exclusion Culture Reduces Campylobacter jejuni Colonization in Broiler Chickens at Slaughter Age In Vivo. Vet Sci. 2022;9.

Taheri HR, Moravej H, Tabandeh F, Zaghari M, Shivazad M. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. Poult Sci. 2009;88:1586–93.

Tamburini, S., Shen, N., Wu, H.C. and Clemente, J.C. 2016 The microbiome in early life: implications for health outcomes. Nat. Med.. 22(7): 713-722.

Tanaka, M. and Nakayama, J. 2017. Development of the gut microbiota in infancy and its impact on health in later life. Aller. Inter. 55:515-522.

Tang, Y., N. Foster, M. A. Jones, and P. A. Barrow. 2018. Model of persistent Salmonella infection: Salmonella enterica serovar Pullorum modulates the immune response of the chicken from a Th17-type response towards a Th2-type response Response. Infect. Immun. 86:1–20. doi:10.1128/IAI.00307-18.

Teng P-Y, Kim WK. Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. Front Vet Sci. 2018;5.

The National Chicken Council.

Thiam M, Wang Q, Barreto Sánchez AL, Zhang J, Ding J, Wang H, et al. Heterophil/Lymphocyte Ratio Level Modulates Salmonella Resistance, Cecal Microbiota Composition and Functional Capacity in Infected Chicken. Front Immunol. 2022;13.

Thomas M, Wongkuna S, Ghimire S, Kumar R, Antony L, Doerner KC, et al. Gut Microbial Dynamics during Conventionalization of Germfree Chicken. mSphere. 2019;4.

Tokuda G, Mikaelyan A, Fukui C, Matsuura Y, Watanabe H, Fujishima M, et al. Fiber-associated spirochetes are major agents of hemicellulose degradation in the hindgut of wood-feeding higher termites. Proc Natl Acad Sci U S A. 2018;115:E11996–2004.

Torok VA, Allison GE, Percy NJ, Ophel-Keller K, Hughes RJ. Influence of Antimicrobial Feed Additives on Broiler Commensal Posthatch Gut Microbiota Development and Performance. Appl Environ Microbiol. 2011;77:3380–90.

Torok VA, Hughes RJ, Ophel-Keller K, Ali M, MacAlpine R. Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult Sci. 2009;88:2474–81.

Turnbaugh, P. J., R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis, and J. I. Gordon. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 444:1027–1031. doi:10.1038/nature05414.

Ulrich-Lynge, S.L., Dalgaard, T.S., Norup, L.R., Kjærup, R.M., Olsen, J.E., Sørensen, P. and Juul-Madsen, H. R. 2015. The consequence of low mannose-binding lectin plasma concentration in relation to susceptibility to Salmonella Infantis in chickens. Vet. Immunol. Immunopathol. 163:23–32.

Ushida, K., Tsuchida, S., Ogura, Y., Toyoda, A. and Maruyama, F. 2016. Domestication and cereal feeding developed domestic pig-type intestinal microbiota in animals of suidae. Anim. Sci. J. 87:835–841.

Val-Laillet, D., Besson, M., Guérin, S., Coquery, N., Randuineau, G., Kanzari, A., Quesnel, H., Bonhomme, N., Bolhuis, J.E., Kemp, B., Blat, S., Le Huërou-Luron I. and Clouard, C. 2017. A maternal Western diet during gestation and lactation modifies offspring's microbiota activity, blood lipid levels, cognitive responses, and hippocampal neurogenesis in Yucatan pigs. FASEB J. 31(5):2037-2049

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T. P., Teillant, A., and Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. 112:5649–5654.

van Heel AJ, de Jong A, Montalbán-López M, Kok J, Kuipers OP. BAGEL3: Automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides. Nucleic Acids Res. 2013;41

van Kuijk SJA, Han Y, Garcia-Ruiz AI, Rodiles A. Hydroxychloride trace minerals have a positive effect on growth performance, carcass quality and impact ileal and cecal microbiota in broiler chickens. J Anim Sci Biotechnol. 2021;12.

Varriale L, Coretti L, Dipineto L, Green BD, Pace A, Lembo F, et al. An Outdoor Access Period Improves Chicken Cecal Microbiota and Potentially Increases Micronutrient Biosynthesis. Front Vet Sci. 2022;9.

Videnska P, Sedlar K, Lukac M, Faldynova M, Gerzova L, Cejkova D, et al. Succession and replacement of bacterial populations in the caecum of egg laying hens over their whole life. PLoS One. 2014;9.

Vineetha, P.G., Tomar, S., Saxena, V.K., Kapgate, M., Suvarna, A.and Adil.,K. 2017. Effect of laboratory-isolated Lactobacillus plantarum LGFCP4 from gastrointestinal tract of guinea fowl on growth performance, carcass traits, intestinal histomorphometry and gastrointestinal microflora population in broiler chicken. J. Anim. Physiol. Anim. Nutr. (Berl). 101:e362–e370.

Volf, J., O. Polansky, K. Varmuzova, L. Gerzova, Z. Sekelova, M. Faldynova, V. Babak, M. Medvecky, A. L. Smith, B. Kaspers, P. Velge, and I. Rychlik. 2016. Transient and prolonged response of chicken cecum mucosa to colonization with different gut microbiota. PLoS One. 11:1–18. doi:10.1371/journal.pone.0163932.

Vonaesch P, Anderson M, Sansonetti PJ. Pathogens, microbiome and the host: Emergence of the ecological Koch's postulates. FEMS Microbiology Reviews. 2018;42:273–92.

Waititu, S. M., A. Yitbarek, E. Matini, H. Echeverry, E. Kiarie, J. C. Rodriguez-Lecompte, and C. M. Nyachoti. 2014. Effect of supplementing direct-fed microbials on broiler performance, nutrient digestibilities, and immune responses. Poult. Sci. 93:625–635. doi:10.3382/ps.2013-03575.

Walter J, Britton RA, Roos S. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the Lactobacillus reuteri paradigm. Proc Natl Acad Sci U S A. 2011;108 SUPPL. 1:4645–52.

Wang B., Yao M., Lv L., Ling Z.,Li L. 2017. The human microbiota in health and disease. Engineering 3:71-82.

Wang J, Fan H, Han Y, Wei J, Zhao J, Zhou Z. Pyrosequencing of the broiler chicken gastrointestinal tract reveals the regional similarity and dissimilarity of microbial community. Can J Anim Sci. 2017;97:302–13.

Wang S, Fan Y, Brunham RC, Yang X. IFN-γ knockout mice show Th2-associated delayed-type hypersensitivity and the inflammatory cells fail to localize and control chlamydial infection. Eur J Immunol. 1999;29:3782–92.

Wang, P., Geng, H., Bu, H.F., Liu F., Wang, X., Kurowski, J., Akhtar, S., Li, Y.Q., and Tan X.D. 2015. Down-Regulation of Peripheral Myelin Protein 22 (PMP22) Contributes to Impairment of Intestinal Barrier Integrity in Sepsis: Role of TNF-α and IFN-γ. Gastroenterology. 148:S-85.

Wei S, Morrison M, Yu Z. 2013. Bacterial census of poultry intestinal microbiome. Poult Sci Sympos. 92:671–83. doi: 10.3382/ps.2012-02822

Wei, H.K., Xue, H.X., Zhou, Z.X., and Peng, J. 2017. A carvacrol-thymol blend decreased intestinal oxidative stress and influenced selected microbes without changing the messenger RNA levels of tight junction proteins in jejunal mucosa of weaning piglets. Animal. 11:193–201.

Wei, S., Gutek, A., Lilburn, M., and Yu, Z. 2013. Abundance of pathogens in the gut and litter of broiler chickens as affected by bacitracin and litter management. Vet. Microbiol. 166:595–601.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, https://ggplot2.tidyverse.org.

Wienemann T, Schmitt-Wagner D, Meuser K, Segelbacher G, Schink B, Brune A, et al. The bacterial microbiota in the ceca of Capercaillie (Tetrao urogallus) differs between wild and captive birds. Syst Appl Microbiol. 2011;34:542–51.

Willing, B. P., and Van Kessel, A.G. 2007. Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. J. Anim. Sci. 85:3256–3266. doi:10.2527/jas.2007-0320.

Willing, B.P., Russell, S.L., and Finlay, B.B. 2011b. Shifting the balance: Antibiotic effects on host-microbiota mutualism. Nat. Rev. Microbiol. 9:233–243.

Willing, B.P., Vacharaksa, A., Croxen, M., Thanachayanont, T., and Finlay, B.B.. 2011b. Altering host resistance to infections through microbial transplantation. PLoS One. 6:2–10.

Winter, S.E., Winter, M.G., Xavier, M.N., Thiennimitr, P., Poon, V., Keestra, A.M., Laughlin, R.C, Gomez, G., Wu, J., Lawhon, S.D, Popova, I.E., Parikh, S.J., Adams, L.G., Tsolis, R.M. Stewart, V.J. and A.J. Bäumler. 2013. Host-derived nitrate boosts growth. Science. 339:708–711.

Wlodarska, M., Willing, B., Keeney, K.M., Menendez, A., Bergstrom, K.S., Gill, N., Russell, S. L., Vallance, B.A., and Finlay B.B. 2011. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated Citrobacter rodentium-induced colitis. Infect. Immun. 79:1536–1545.

Wongkuna S, Kumar R, Ghimire S, Mattiello Drescher S. Identification of a microbial sub-community from the feral chicken gut that reduces Salmonella colonization and improves gut health in a gnotobiotic chicken model. bIOrXiv. 2022.

Wood-Gush DGM. A History of the Domestic Chicken from Antiquity to the 19th Century.

Xi Y, Shuling N, Kunyuan T, Qiuyang Z, Hewen D, ChenCheng G, et al. Characteristics of the intestinal flora of specific pathogen free chickens with age. Microb Pathog. 2019;132:325–34.

Xie, H., N. C. Rath, G. R. Huff, W. E. Huff, and J. M. Balog. 2000. Effects of Salmonella typhimurium lipopolysaccharide on broiler chickens. Poult. Sci. 79:33–40. doi:10.1093/ps/79.1.33.

Xu Y, Yang H, Zhang L, Su Y, Shi D, Xiao H, et al. High-throughput sequencing technology to reveal the composition and function of cecal microbiota in Dagu chicken. BMC Microbiol. 2016;16:1–9.

Yadav S, Caliboso KD, Nanquil JE, Zhang J, Kae H, Neupane K, et al. Cecal microbiome profile of Hawaiian feral chickens and pasture-raised broiler (commercial) chickens determined using 16S rRNA amplicon sequencing. Poult Sci. 2021;100.

Yan H., Lu H., Almeida, H.H., Ward, M.G., Adeola, O., Nakatsu, C.H., and Ajuwon K.M. 2015. Effects of dietary resistant starch content on metabolic status, milk composition, and microbial profiling in lactating sows and on offspring performance. J. An. Phys. Nut. 1-11.

Yan L. 2022. ggvenn: Draw Venn Diagram by 'ggplot2'. R package version 0.1.9.

Yan, C., J. Xiao, Z. Li, H. Liu, Xinjie Zhao, J. Liu, S. Chen, and Xingbo Zhao. 2021. Exogenous fecal microbial transplantation alters fearfulness, intestinal morphology, and gut microbiota in broilers. Front. Vet. Sci. 8:1–11. doi:10.3389/fvets.2021.706987.

Yang L, Chen J. A comprehensive evaluation of microbial differential abundance analysis methods: current status and potential solutions. Microbiome. 2022;10.

Yang Q, Ren S, Niu T, Guo Y, Qi S, Han X, et al. Distribution of antibiotic-resistant bacteria in chicken manure and manure-fertilized vegetables. Environmental Science and Pollution Research. 2014;21:1231–41.

Yang, L., Liu, S., Ding, J., Dai, R., He, C., Xu, K., Honaker C.F., Zhang, Y., Siegel, P. and Meng H. 2017. Gut microbiota co-microevolution with selection for host humoral immunity. Front. Microbiol. 8:1–11.

Yeoman CJ, Chia N, Yildirim S, Berg Miller ME, Kent A, Stumpf R, et al. Towards an evolutionary model of animal-associated microbiomes. Entropy. 2011;13:570–94.

Yeoman, C. J., N. Chia, P. Jeraldo, M. Sipos, N. D. Goldenfeld, and B. A. White. 2012. The microbiome of the chicken gastrointestinal tract. Anim. Health Res. Rev. 13:89–99. doi:10.1017/S1466252312000138.

Yokota H, Coates ME. The uptake of nutrients from the small intestine of gnotobiotic and conventional chicks. British Journal of Nutrition. 1982;47:349–56.

Yoon SH, Ha S min, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology. 2017;110:1281–6.

Yoshikawa, S., R. Araoka, Y. Kajihara, T. Ito, H. Miyamoto, and H. Kodama. 2018. Valerate production by Megasphaera elsdenii isolated from pig feces. J. Biosci. Bioeng. 125:519–524. doi:10.1016/j.jbiosc.2017.12.016.

Youssef, N.H., Couger, M.B., McCully, A.L., Criado, A.E.G., and Elshahed, M.S. 2015. Assessing the global phylum level diversity within the bacterial domain: A review. J. Adv. Res. 6:269–282.

Zaytsoff et al., S. J. M., T. Montina, V. F. Boras, J. Brassard, P. E. Moote, R. R. E. Uwiera, and G. D. Inglis. 2022. Microbiota transplantation in day-old broiler chickens ameliorates necrotic enteritis via modulation of the intestinal microbiota and host immune responses. Pathogens. 11. doi:10.3390/pathogens11090972.

Zenner, C., T. C. A. Hitch, T. Riedel, E. Wortmann, S. Tiede, E. M. Buhl, and B. Abt. 2021. Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. mSystems. 6:e01300-20. https://doi.org/10.1128/mSystems.01300-20.

Zgheib R, Anani H, Meng MM, Mailhe M, Ricaboni D, Morand A, et al. New human-associated species of the family atopobiaceae and proposal to reclassify members of the genus olsenella. Int J Syst Evol Microbiol. 2021;71.

Zheng, L., Kelly, C.J., Battista, K.D., Schaefer, R., Lanis, J.M., Alexeev, E.E., Wang, R.X., Onyiah, J.C., Kominsky, D.J. and Colgan, S.P. 2017. Microbial-Derived Butyrate Promotes Epithelial Barrier Function through IL-10 Receptor–Dependent Repression of Claudin-2. J. Immunol. ji1700105. doi:10.4049/jimmunol.1700105.

Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., De Sauvage, F.J. and Ouyang, W. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat. Med. 14:282–289.

Zhu, A., Ibrahim, J. G., Love, M. I. 2019. Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences, Bioinformatics, 35(12):2084–2092, https://doi.org/10.1093/bioinformatics/bty895

Zilber-Rosenberg I, Rosenberg E. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. FEMS Microbiology Reviews. 2008;32:723–35.