Université de Montréal

Dynamics of the fecal microbiota of veal calves after arrival to a rearing unit

Par

Sarah Elizabeth Izzo Crespo

Mémoire présenté à la Faculté de médecine vétérinaire en vue de l'obtention du grade de *Maîtrise es sciences* en sciences vétérinaires, option biomédecine

Octobre 2023

Université de Montréal

Département de biomédecine, Faculté de médecine vétérinaire

Ce mémoire intitule

Dynamics of the fecal microbiota of calves after arrival to a rearing unit

Présenté par

Sarah Elizabeth Izzo Crespo

A été évaluée par un jury composé des personnes suivantes

Réjean Lefebvre
Président-rapporteur

Márcio Carvalho Costa
Directeur de recherche

Gilles Fecteau
Codirecteur

Mélissa Duplessis
Membre du jury

Résumé

Le microbiote gastro-intestinal joue un rôle important dans le maintien de la santé de l'hôte. Il est composé de nombreux micro-organismes tels que des bactéries, des virus, des champignons et des archées. Cependant, la majorité de ces cellules microbiennes sont des cellules bactériennes et, pour cette raison, de nombreuses études se concentrent sur l'exploration des communautés bactériennes en particulier dans le tube gastro-digestif. Un déséquilibre de cette microbiote, appelé dysbiose, a été observé dans plusieurs pathologies telles que la diarrhée, la pneumonie, après l'administration d'antibiotiques ou une modification du régime alimentaire. L'objectif de cette étude était de caractériser la dynamique du microbiote fécal des veaux entrant dans une unité d'élevage. Cinquante veaux Holstein âgés de 8 à 14 jours et arrivant dans une unité de veaux ont été inscrits à cette étude. Des échantillons fécaux ont été collectés à l'arrivée et les jours 4, 10 et 24 après l'arrivée. Les scores fécaux, le poids des veaux et l'administration d'antibiotiques ont été enregistrés au cours de l'étude. Le séquençage a été réalisé à l'aide de la plateforme Illumina MiSeq et les données analysées à l'aide du logiciel Mothur. Contrairement aux attentes, la richesse et la diversité étaient plus élevées lorsque la proportion d'animaux diarrhéiques était plus élevée ($p < 0,001$) et, comme prévu, la composition et la structure du microbiote changeaient au fil des jours de collecte ($p > 0,001$), mais les changements n'étaient pas associés à présence ou non de diarrhée et de traitement antibiotique comme prévu, ils sont associés aux jours de prélèvement. La proportion de diarrhée (nombre de veaux diarrhéiques par jour) était numériquement plus élevée les jours 4, 10 et 24 après l'arrivée. Comme prévu, les abondances relatives de bactéries associées à la santé (par example : *Bifidobacterium, Lactobacillus* et *Faecalibacterium*) ont diminué chez les veaux diarrhéiques. Bien que l'analyse de la diarrhée et de l'utilisation d'antibiotiques ne fît pas partie des objectifs de cette étude, il y avait une tendance ($p=0,09$) dans le poids des animaux ayant eu la diarrhée et ayant reçu des antibiotiques. Le poids final des veaux malades ayant reçu des antibiotiques avant l'abattage étaient inférieurs par rapport au poids final des animaux qui n'étaient pas malades et n'avaient pas reçu d'antibiotiques ($p=0,072$). La principale limite de cette étude est le manque d'information sur l'origine des veaux avant leur arrivée à l'unité d'élevage. Cette étude contribue à une meilleure compréhension des

changements microbiens liés au stress auquel sont confrontés les veaux de boucherie et pourrait servir de base à d'autres études visant à proposer des méthodes alternatives de manipulation du microbiote pour prévenir les maladies et rétablir la santé des veaux.

Mots clés : Diarrhée, microbiote fécal, microbiome, dysbiose, microbiote gastro-intestinal des veaux, séquençage de l'ADN, stress.

Abstract

The gastrointestinal microbiota plays an important role in maintaining the health of the host. It is composed of many microorganisms such as bacteria, viruses, fungi, and archaea. However, the majority of these microbial cells are bacterial cells, and for that reason, many studies focus on exploring especially bacterial communities in the GIT. Imbalance of the GIT microbiota, termed dysbiosis, has been observed in several conditions such as diarrhea, pneumonia, after antibiotic administration, or diet modification. The objective of this study was to characterize the dynamics of the fecal microbiota of veal calves entering a rearing unit. Fifty Holstein calves ranging from 8-14 days of life and arriving in a veal unit were enrolled in this study. Fecal samples were collected on arrival and on days 4 ,10 and 24 after arrival. Fecal scores, calves' weight and antibiotic administration were recorded during the study. Sequencing was performed using the Illumina MiSeq platform and data analysed using the software Mothur. Contrary to expectations, richness and diversity were higher when the proportion of diarrheic animals were higher ($p<0.001$), and as expected, the microbiota composition and structure changed among days of collection ($p>0.001$), but the changes were not associated to presence or absence of diarrhea and antibiotic treatment as expected, they are associated to the sampling days. Diarrhea proportion (number of diarrheic calves per day) were numerically higher on days 4, 10 and 24 after arrival (As expected, the relative abundances of bacteria associated to health (i.e., *Bifidobacterium, Lactobacillus and Faecalibacterium)* were decreased in the diarrheic calves. Although analyzing diarrhea and antibiotic usage was not one of the objectives of this study, there was a tendence ($p=0.09$) in the weigh of the animals that had diarrhea and received antibiotics. The final weight of the sick calves that received antibiotics before slaughter were lower when compared to the final weigh of the animals that were not sick and did not received antibiotics ($p=0.072$). The main limitation of this study is the lack of information about calves' origin before arrival at the rearing unit. This study contributes to the better understanding of the microbial changes related to the stress faced by veal calves and might be the basis for further studies to propose alternative methods of microbiota manipulation to prevent disease and restore health in calves.

Keywords: Diarrhea, fecal microbiota, microbiome, dysbiosis, Calves gastrointestinal microbiota, DNA sequencing, stress.

Table of contents

Résumé 3

Abstract 5

Table of contents 7

List of figures 9

List of Acronyms and Abbreviations 10

Acknowledgements 12

Introduction 13

Chapiter 1 – Literature review 15

1.1 DNA sequencing 15

1.2 Concepts used in the analyze of bacterial populations 17

1.3 What is microbiota and its importance 17

1.4 Colonization of the intestinal tract 18

1.5 Factors that can change the GIT microbiota 20

1.5.1 Age 20

1.5.2 Diet 20

1.5.3 Genetics 22

1.5.4 Environment (farming practices) 23

1.5.5 Antibiotic exposure 24

1.6 Dysbiosis 25

1.7 Conclusions 27

Chapiter 2 – Scientific manuscript 28

Dynamics of the fecal microbiota of veal calves after arrival to a rearing unit 28

Abstract 29
Introduction 30
Materials and Methods 31
Animals and Sample Collection 31
Ethics statement 32
DNA extraction 32
16S rRNA gene amplicon sequencing 32
Bioinformatic analysis 33
Results 33
Microbiota analysis 33
Alpha Diversity 35
Principal Coordinate Analysis (PCoA) 36
Relative Abundances 38
Linear discriminant analyses (LDA) Effect Size (LEfSe) 41
Discussion 41
Conclusions 43
References 45
Chapter 3 - General discussion 59
References 62

List of figures

Figure 1. – Diarrhea incidence according to sampling days (D0, D4, D10 and D24 – AMOVA – Tuckey test)….. 34

Figure 2. – Comparation of the average weight (kg) of animals that had diarrhea or not during the study. Initial weight (auction exit) was used as a covariant (ANCOVA - Analyze de covariance; p=0.072)……35

Figure 3. – Chao richness estimator, Simpson's diversity index, and Shannon index for feces on each one of 4 sampling days (D0, D4, D10 and D24). Different letters indicate significant differences (Chao1 $p < 0.001$; Inv. Simpson $p < 0.01$ and Shannon, $p < 0,001$). 35

Figure 4. – one-way ANOVA). Bars represent means. ..... **Erreur ! Signet non défini.**

Figure 5. – Principal coordinate analysis (PCoA) of bacterial communities' membership showing the microbiota dynamics over time. 37

Figure 6. – Principal coordinate analysis (PCoA) of bacterial communities' membership of healthy and diarrheic calves (A) and structure of healthy and diarrheic (B) calves present in feces on days D0, D4, D10 and D24.....38

Figure 7. – Relative abundances of the main taxa (1% abundance) present in the calves' feces according to the days of sampling (0, 4 10 and 24), at phylum level. 39

Figure 8. – Relative abundances of predominant bacteria present in the calves' feces according to the days of sampling (0, 4 10 and 24) and healthy status (healthy or diarrheic), at genus level. The eighteen most abundant genera are represented.....40

Figure 9. – Lefse Predicted functional composition of metagenomes based on 16S rRNA gene sequencing data revealed differentially enriched bacterial functions associated to sampling days……. 41

List of Acronyms and Abbreviations

AGP : Antibiotic Growth Promoters

ASV : Amplicon Sequencing Variants

GI : Gastrointestinal

GIT : Gastrointestinal tract

OTUs : Operational Taxonomic Unit

To my mother and father for all the sacrifices made to get me here

Acknowledgements

To my director **Dr. Marcio Costa** and my codirector **Dr. Gilles Fecteau**, I am grateful for the opportunity and your assistance.

To my council committee **Dr. Gustavo Zamberlam** and **Dr. Sébastien Buczinski** for all tips and assistance. Thank you for being part of my council committee.

To the **members of my jury**, thank you for accepting to review my mémoire during these demanding times.

To **Rebecca Di Pietro, Laura Franco, Augusto Holken, and Jean Ramos** thank you for your incessant help and encouragement throughout this adventure.

To **Hector Salgado** and **Amal Roussi**, thank you for your friendship, your corrections and for making me laugh even when I want to cry.

To **Jefo team,** for my job, the welcoming environment, the coffees, and the happy moments in the last year.

To **Nicola Bousquet**, my life partner, thank you for your endless love and your efforts to be patient. Thank you for Thomas and the family that we have started together. Thank you for the calm that you give me.

To **Christian Ledoux**, thank you for taking care of Nic and for helping me to endure him (hi).

To my **lovely family**, thank you for your words of encouragement and constant interest in my life. Thank you, mom, for always supporting my dreams.

To **Sophia Bilodeau, Christine Blondin** and **Eveline Malo,** thank you for your patience and willingness to always help me.

Introduction

The gastrointestinal (GI) microbiota is a group of microorganisms that play an essential role in maintaining the health of the host. The gastrointestinal tract (GIT) microbiota is composed of bacteria, archaea, protozoa, fungi (mostly yeasts), and viruses (Barko et al., 2018). More than 98% of genetic sequences present in the GIT come from bacteria, the focus of most research in this area refer to the intestinal bacteria and their interaction with the host (Jandhyala et al., 2015), and for this reason, the term GIT microbiota frequently refers to the GIT bacteria.

The GIT microbiota is involved in nutrient digestion and uptake, synthesis of volatile fatty acids, amino acids, vitamins, maintenance of intestinal mucosal integrity and GIT peristalsis, as well as aiding in development of the enteric immune system, including epithelial secretion of antimicrobial peptides (Sommer & Bäckhed, 2013). Hence, the microbiota helps to protect the body from pathogenic organisms by competing for defined metabolites and attachment sites, which can significantly affect the expression of pathogen virulence genes and bacterial growth rates (Kamada et al., 2013).

It is assumed that the ruminants are born with a sterile gastrointestinal tract and the initial microbial colonization starts directly after birth through the passage of the newborn by the vaginal canal (Dominguez-Bello et al., 2010). Maternal contact, environmental exposure and feed are the initial sources of microbiota during the immediate neonatal period.

At a very young age, veal calves are faced with major stress events like transportation, marketing, dietary changes, and exposure to a variety of infectious agents, which can contribute to decreasing the protective potential of the GIT microbiota (Timmerman et al., 2005). In the presence of these stress events, changes in the microbial community composition (dysbiosis) are associated with an increase in potentially pathogenic bacteria and a decrease in health-associated bacteria (Levy et al., 2017).

In summary, the characterization of the pre-weaning calf's GIT microbiota is very important to understand the establishment of the normal microbiota. This knowledge should help

to understand and define dysbiosis status in order to develop strategies to maintain and/or restore a healthy microbiota under different farm practices.

Chapiter 1 – Literature review

1.1 DNA sequencing

Culture-based methods have been used to identify microbial organisms in predetermined culture medium under controlled conditions. However, there is no single culture medium that can support growth of all the bacterial species of the gastrointestinal tract (GIT). Only 5-20% of the bacteria can be cultured in traditional culture media (Costa & Weese, 2019). For example, approximately 62% of bacteria from the human intestine were previously unknown, and 80% of bacteria identified (by DNA sequencing) were difficult to grow (Eckburg et al., 2005). Culture-based methods can also overestimate the significance of bacterial species that grow extremely well in selective and enriched media conditions. Therefore, the classification of GIT bacteria based on phenotypic characteristics and biochemical tests is not sufficient to study the diversity among the organisms present in the gastrointestinal tract (Costa & Weese, 2019).

Sequencing-based methods for bacterial identification have emerged as an attractive strategy for the study of complex bacterial environments. Ribosomal gene sequences are commonly used for bacterial identification and phylogenetic analyses. The 16S small ribosomal subunit (16S rRNA) gene is widely used for taxonomic classification because the gene is ubiquitous and unique to each bacteria and archaea (Kolbert & Persing, 1999; Mizrahi & Jami, 2018). The 16S rRNA gene is approximately 1.5kb long, however, like most genes, it contains regions of conserved and variable sequences. The more conserved regions are used to pinpoint the gene and design primers for amplification of the variable regions, used for species identification. The 16S rRNA gene contains nine hypervariable regions (V1-V9), with lengths varying from approximately 50 bases to 254 bases, each flanked by highly conserved regions. Universal primers designed for the conserved regions are used to amplify these hypervariable regions. A sequence off one or more hypervariable regions, such as V2 and V3, is sufficient to distinguish bacteria at the genus level (Mizrahi-Man et al., 2013; Weisburg et al., 1991). Analysis of the entire 16S gene sequence can even distinguish between species and sometimes strains (Kolbert & Persing, 1999; Weisburg et al., 1991). Sequencing of the V4 region compared to other regions demonstrated a better cost-

benefit ratio, with good specificity in the classification of organisms and suitable estimates of richness and diversity (Mizrahi-Man et al., 2013).

Sanger sequencing was the first sequence-based method used to evaluate mixed microbial populations. This provided revolutionary new information but was limited to the sequencing of one DNA strand at a time. Studies that identified only a few hundred DNA sequences were expensive and time-consuming, yet the data they provided was unprecedented and showed the value of a sequence-based approach. The Sanger (capillary-based) sequencing approach has been replaced by high throughput DNA sequencing technologies (Mardis, 2013).

The deployment of by high throughput DNA sequencing technologies has provided greatly enhanced capabilities for sequencing large meta-datasets. Technology has created new opportunities for the pursuit of large-scale sequencing projects. One example is the Illumina platform which consists of lengthening the sequence by synthesis, in which a luminous signal with different coloring is emitted at the time of incorporation of each nucleotide (Klindworth et al., 2013)

Compared with Sanger technologies, by high throughput DNA sequencing technologies platforms offer the combined advantages of speed, automation, high throughput, and lower cost per read. Hence, with the technological advances in recent years, it is possible to identify the main taxonomic groups present in various environments and to verify the changes in proportions of these organisms. With the launch of the Illumina platform, the cost in relation to the number of bases used and the number of strings became lower in relation to other platforms (Bentley et al., 2008). However, we must consider the size of the fragment to be sequenced, the complexity of the community, as well as the type of study. The advancements in high-throughput sequencing in parallel with their decreasing costs reveal how crucial is the microbiota in the understanding of health and disease.

1.2 Concepts used in the analyze of bacterial populations

Some concepts about microbiota must be known for a better understanding and interpretation of the results that refer to the GIT bacterial communities. The term "taxon" is used to indicate an unit at any level of a taxonomic classification system: kingdom, phylum, class, order, family, genus, and species. It is used when the taxonomic ranking is not specified (Costa & Weese, 2019). Richness is a direct count or estimate of the number of different species of bacteria present in an environment or in a community. The manner these species are distributed in the communities determines their evenness (i.e., how equal the community is distributed). Diversity is a mathematical calculation that takes into account the richness and the evenness of a community (Costa & Weese, 2019; Valdes et al., 2018).

Alpha-diversity analysis refers to the individual characteristics of each community (richness and diversity). Beta-diversity attributes a taxonomic classification to those organisms and is used to compare the composition of microbial communities. When only presence or absence or each taxon is used to compare the shared species between communities, it is called membership, but if the abundance of each species is also considered, the analysis is called community structure (Costa & Weese, 2019; Lozupone & Knight, 2005).

The term relative abundance is commonly used to express the percentages of each bacterial population in relation to the abundances of all other members of the community. This is not a measure of absolute abundance, since the actual bacteria count is lost during the PCR amplification process (Rajendhran & Gunasekaran, 2011).

Operational taxonomic unit (OTU) is a definition used to classify groups of closely related sequencing reads and is normally defined in the setting of bioinformatic parameters. For example, sequences grouped into OTUs with 97% similarity (Costa & Weese, 2019).

1.3 What is microbiota and its importance

Cattle provide milk and meat to meet growing demands for animal proteins as the human population increases. In the rumen, some dietary substrates that are unsuitable for human

consumption can be converted into high-quality animal protein through the symbiosis that exists between the host and the microorganisms present in their GIT (Eisler et al., 2014).

The gastrointestinal microbiota is composed of microorganisms such as bacteria, archaea, protozoa, fungi (mostly yeasts), and viruses (Costello et al., 2012; Dominguez-Bello et al., 2010).

These microorganisms are involved in nutrient digestion and uptake, synthesis of volatile fatty acids, amino acids, vitamins, maintenance of intestinal mucosal integrity and GIT peristalsis. In addition,, they help the development of the enteric immune system including epithelial secretion of antimicrobial peptides and the local immunomodulation on the tight junctions (Sommer & Bäckhed, 2013). At the same time, the microbiota helps to protect the body from pathogenic organisms by competing for defined metabolites and attachment sites, which can significantly affect the expression of pathogen virulence genes and bacterial growth rates (Kamada et al., 2013).

In calves, the microorganisms present in the GIT are capable of converting some dietary substrates that are unsuitable for human consumption into high-quality animal protein (Eisler et al., 2014). These microbiota is also involved in the synthesis of vitamins (B group and K), enhancement of GIT motility and function and metabolism of plant compounds/drugs (Cani et al., 2019; Malmuthuge, Griebel, et al., 2015; Tan et al., 2014).

To better analyse the importance of these microorganisms and how they reach the GIT and are capable to colonising it, the early colonisation of the calf's GIT must be understood.

1.4 Colonization of the intestinal tract

The gastrointestinal tract of most animals before delivery is sterile (Kelly et al., 2007; Mackie et al., 1999; Maynard et al., 2012; Tizard, 1993), therefore, the bacterial colonization of the gastrointestinal tract of the newborn starts at the moment of birth (Klein-Jöbstl et al., 2019; Maynard et al., 2012). After birth, the intestinal microbiota goes through rapid changes of bacterial composition, diversity and abundances, before reaching adulthood where the microbiota is relatively stable (Dias et al., 2018; Yeoman et al., 2018).

After the first day of life some bacteria are not true colonisers but only transient organisms. As the transient organisms subside, the true colonisers start to take hold (Costa et al.,

2015). Multiple environmental factors, including diet, exposure to new microbes, and intestinal infections, play important roles in shaping the composition of the microbiota during this maturational window (Maynard et al., 2012).

The composition of the microbiota of calves changes substantially at 2 stages in early life: from birth to weaning (6-8 weeks of age), and from weaning to adulthood (Klein-Jöbstl et al., 2019; Uyeno et al., 2010). During the first 3 weeks of life, significant changes in anatomy and physiology of the GIT occur and immediately afterwards the pre-weaning period is associated with great stress, metabolic and immunologic challenge to calves (Klein-Jöbstl et al., 2019; Malmuthuge et al., 2013).

The rich but low diverse microbiota in calves' changes with the acquisition of the bacteria present in the environment. The calf GIT microbiota during the first two days of life is distinct from the dam's faecal microbiota (Dill-Mcfarland et al., 2017; Kim et al., 2014; Ozutsumi et al., 2005). Although some bacteria are constantly present during the development phase, others inhabit the intestine only for short periods. The newborn GIT is first colonized by facultative anaerobes (Jami et al., 2013; Li et al., 2019), which then create the anaerobic conditions required for colonization by obligate anaerobic bacteria, such as *Bifidobacterium* spp. and *Bacteroides* (known to be related to digestion of milk) (Malmuthuge et al., 2019) . These changes reflect the gradual adaptation of the calf GIT first to milk consumption and later to consumption of solid feed. This finding is not surprising as a newborn calf is fed by liquid feed only and has not developed a functional rumen (Alipour et al., 2018; Klein-Jöbstl et al., 2019). At six months of age their microbiota is similar to the adult's microbiota.

The newborn calf rectal microbiota is dominated by Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes (Alipour et al., 2018; Malmuthuge & Guan, 2017; Rey et al., 2014) and individual variations observed among calves decrease with calf age (Klein-Jöbstl et al., 2014).

1.5 Factors that can change the GIT microbiota

The composition and diversity of the GIT microbiota may be attributed to various factors such as age, diet, environment, genetics, antibiotic treatment during early life, and many more identified or unidentified factors (Choudhury et al., 2015; Hasan & Yang, 2019).

1.5.1 Age

In humans, the microbiota undergoes the most prominent changes during infancy and the elderly. Interestingly, the immune response is also at its weakest point and most unstable state during these two critical stages of life (Nagpal et al., 2018). Contact with environments of greater bacterial diversity at a young age is related to the maturation of the immune system and lower incidence of diseases during childhood (Kuo et al., 2013).

The GIT of the calf is considered sterile before birth (Govil et al., 2017; Li et al., 2012). At birth, the pioneer bacterial populations colonizing calves within the first 48 hours of life are facultative aerobes with high abundances of Bacteroidetes. With advancing age and weaning, bacteria belonging to the phylum Firmicutes become the most abundant within the GIT (Jami et al., 2013) and at the same time, the relative abundances of some bacteria such as those of the genera *Lactobacillus* and *Bifidobacterium* decrease (Uyeno et al., 2010). Calves will only develop a stable GIT microbiota resembling the one of adult around six months of age (Amin & Seifert, 2021).

1.5.2 Diet

The diet is probably the most important factor influencing the abundance of the different species in the GIT and could serve as a tool to alter the microbiota in early life. The ecosystem is dynamic as the microbial population changes considerably with diet. Substrate availability to microbial populations and new ingredients drive the populational changes (Choudhury et al., 2015; Henderson et al., 2015), and the proportions of the different species in the GIT will shift to a new balance (Kim et al., 2014; Li et al., 2019).

The calf is functionally a monogastric at 7 days of age, the rumen is not fully developed until 12 weeks after birth (Hartinger et al., 2022). At birth, the pre-stomachs of the ruminant are

non-functional, undeveloped, and disproportionate to the adult digestive system. The transition from a monogastric to ruminant animal requires the development of the reticule-rumen and its associated with the development of the microbial population for efficient utilization of dry and forage-based diets (Heinrichs, 2005).

The importance of an adequate colostrum supply in the first hours of life, and therefore the passive transfer of immunoglobulins for calves' health is well known. Time of first colostrum feeding, as well as colostrum quantity and quality, plays an important role in the calf's health (Klein-Jöbstl et al., 2014). Colostrum is not only a source of energy and immunoglobulins as IgG but can also be a source of bacteria for the early establishment of the GIT microbiota. It has been shown that feeding colostrum soon after birth enhances the colonization of total bacteria in the gastrointestinal tract of calves within the first 12 h compared with calves not given colostrum (Malmuthuge, Griebel, et al., 2015). In addition, studies have shown that fed heat-treated colostrum may serve as prebiotic to microbiota in the intestine of the neonatal calf and delaying colostrum feeding within 12 h of life delay the colonization of bacteria in the intestine, possibly leaving the calf vulnerable to infections during the pre-weaning period (Malmuthuge et al., 2015; Shams et al., 2022). Fresh colostrum may contain *Lactobacillus, Bifidobacterium, Escherichia* (Malmuthuge et al., 2015), *Staphylococcus* spp., coliforms, and *Streptococcus* spp. (Lima et al., 2017). Thus, the bacterial composition of colostrum can be highly important for microbial colonization of the GIT. Moreover, colostrum contains certain oligosaccharides that are of importance of GIT health by inhibiting the adherence of pathogens to the intestinal epithelial cells or by serving as growth substances for the establishment of a healthy bacterial community (Hang et al., 2021).

Activation of ruminal fermentative processes commences with the introduction of solid feed into the diet, with a dramatic shift occurring when milk is completely removed from the diet (weaning). As the source from which an animal attains its nutrition shifts, greatly altering the composition of the ruminal and intestinal microbiomes (Meale et al., 2016).

A study compared calves raised on low-fiber and high-protein starter grains to those raised on high-fiber corn silage or a mixture of starter grains and corn silage to determine the short- and

long-term impacts of the calf diet. The results showed that silage-fed calves had lower bacterial diversity compared with the other groups. However, among the calf diets, the ruminal microbiota of silage-fed calves at weaning had more bacteria in common with adults indicating that pre-weaning feed has effect on the developing GIT microbiota (Dill-McFarland et al., 2019).

The development of rumen microbiota in calves can directly affect feed intake, nutrient digestibility, and eventually growth. Any changes in the early feeding regime and nutrition can influence rumen development, and thus, lead to long-lasting effects on subsequent growth, health, and milk production performance (Diao et al., 2019). Microbes in the GIT metabolize feedstuffs into VFAs, microbial biomass, vitamins, and other substances for the host's nutritional requirements. Each microbial species has evolved with specific substrate preferences (Malmuthuge, 2016), and the steady supply of food and constant removal of digested feed material and end products allow a dense population of microorganisms to grow in the GIT (Kamra, 2005). GIT microbiota adaptation may take several days or weeks to take place, depending on how drastic a change is made in the diet (Leeming et al., 2019).

1.5.3 Genetics

Interestingly, providing identical feed to the animals in the same herd did not necessarily establish identical microbial composition among individuals, suggesting that host-specific conditions may be an important factor influencing the GIT microbiota. The impact of host genetics may be one of the factors contributing to the high inter-individual variation observed in the GIT microbiomes of neonates, whose diet is not yet stabilized (Malmuthuge et al., 2019).

A study evaluating calves with varying breed composition from 100% Angus (*Bos taurus*) to 100% Brahman (*Bos indicus*) raised in the same environment showed that the GIT microbiota at 3 months of age was significantly affected by host genetics (Fan et al., 2020). In addition, a rumen microbial features were discovered to be heritable and could be influenced by host genetics, highlighting a potential to manipulate and obtain a desirable and efficient rumen microbiota using genetic selection (Li et al., 2019) These findings indicate a strong contribution by host genetics in shaping the GIT microbiota, shedding the light on the impact of animal breeding on microbiota, which is associated with animal growth and health (Fan et al., 2020).

1.5.4 Environment (farming practices)

Environmental factors clearly interact with the acquisition and maintenance of a stable GIT microbiota. Although there are differences in the microbiota of cattle submitted to the same diet and management conditions, the environment in which these animals are inserted (farming practices) can also influence the microbiota (Gomez et al., 2017; Weese & Jelinski, 2017). Calves could be exposed at a very young age to adult-cow-associated microorganisms present in the environment or through contact with humans who interact with adult animals (Dill-Mcfarland et al., 2017).

Interestingly, not only is the animal's GIT microbiota affected by the farming but also the farmer workers microbiota. In Switzerland, close contact with pigs has been shown to affect pig farmers' nasal microbiota (Moor et al., 2021). A recent study in Thailand reinforces that pig and poultry farming are capable of influencing human GIT microbiota composition probably due to greater quantities of and/or exposure to allergens and/or endotoxins, and bioaerosols in animal barns (Sudatip et al., 2022) showing the importance and influence of the environments not only for animals but also for humans who work with animals.

Under typical modern production conditions, dairy calves receive minimal maternal care that can contributes to a decreasing diversity. A recent study showed greater richness and diversity and an earlier establishment of the microbiota in calves raised with their dams compared to calves without their dams (Li et al., 2022).

Previous studies of farm animals have involved single farms or research facilities, precluding an ability to assess inter-farm variation. The majority of the studies compare groups of animals from the same farm or treatments between a few farms (Gomez et al., 2017; Theelen et al., 2021; Weese & Jelinski, 2017) , but the differences between farms suggest that there may be important and currently unidentified management practices that can significantly influence the microbiota and reinforces the necessity of further studies accessing a bigger number of farms and comparisons among animals.

1.5.5 Antibiotic exposure

Antibiotics are among the most frequently prescribed medications during pregnancy and lactation for women and animals and can cause significant shifts in the GIT microbiota resulting in the suppression of both beneficial and pathogenic bacteria (Tejada, 2014). Whereas some of these therapeutic strategies have been shown to be beneficial to reduce short-term maternal and neonatal complications, their long-term effects are by far less understood (Tejada, 2014). It takes weeks to months to return the microbiome populations back to normal following antibiotic treatment, and antimicrobial use is the main driver of antimicrobial resistance (Holmes et al., 2015).

It has been suggested that exposure to antibiotics during fetal/neonatal life affects the development of diseases via their adverse and possible long-term effects on GIT microbiota of both the mother and child, and on the vaginal microbiota of the mother (Tejada, 2014). Antibiotic use may delay and interfere with the early colonization of the human baby's microbiota. In turn, this delay or aberrant colonization may interfere with the development and maturation of the offspring's immune system, and thus play a role in the development of allergies and disease (Jakobsson et al., 2010). Antibiotic use early in life can be associated with the risk of childhood asthma, allergies, and obesity, (Dominguez-Bello et al. 2010) and atopic dermatitis (Bager et al., 2008) in humans.

In calves, the use of antibiotics to treat diarrhea and respiratory disease is common (Gomez et al., 2017). At a very young age, the animals are faced with major stress events like transportation, overpopulation, dietary changes, new environment and exposure to a variety of infectious agents, which can contribute to decrease the protective potential of the GIT microbiota (Timmerman et al., 2005). The effects of antibacterial drugs upon the microbial communities of the GIT are poorly understood in this species.

There are significant differences in microbial diversity between healthy and diarrheic calves within a farm (Gomez et al., 2017), and these differences are more evident especially when comparing animals being treated with antibiotics. A study comparing 38 diarrheic and 45 healthy calves, showed that antimicrobial-treated calves had lower diversity compared to healthy calves.

Although optimal microbial diversity is not well understood, decreased diversity is often associated to dysbiosis and disease, and may lead to a limited ability of the microbiota to respond to different stressors (Weese & Jelinski, 2017).

The GIT microbiota changes in sick calves usually return to the pre-diarrheal stage after a week (Varshney & Naresh, 2005). It is not clear if the reduction in microbial diversity occurs due to the disease itself or due to the antibiotic treatment (Messer & Chang, 2018). A study in 2013 showed that calves preventively treated with antibiotics or fed with medicated milk replacer had 70% and 31% more days with diarrhea, respectively, compared to calves that only received antibiotics in cases of fever and depression (Gibbons et al., 2013).

1.6 Dysbiosis

The term "dysbiosis" was originally coined by Metchnikoff to describe altered pathogenic bacteria in the GIT. Dysbiosis is a state in which the microbiota produces harmful effects via: (1) qualitative and quantitative changes in the intestinal microbiota itself; (2) changes in their metabolic activities; and (3) changes in their local distribution. These factors result in alterations in bacterial metabolism, as well as the overgrowth of potentially pathogenic microorganisms (Bäckhed et al., 2012).

Dysbiosis can be defined as an imbalance in the GIT microbial community composition that is associated with disease. Dysbiosis could cause or contribute to disease in different ways: it could lead to gain of one or more microorganisms with detrimental functions to the host or loss of one or more microorganisms with functions beneficial to the host. Since many of the microbes in the GIT community have important functional relationships with each other, changes in a small number of microbes and/or their functions could have broad impacts on the community (Kostic et al., 2014).

In humans, dysbiosis has been associated with several diseases including inflammatory bowel disease, diabetes, asthma, and obesity (Pelzer et al., 2017). In cattle, dysbiosis is associated with diseases such as bovine respiratory disease, bovine digital dermatitis, mastitis, Johne's disease, uterine diseases (metritis and endometritis), and metabolic disorders (ruminal acidosis

and ketosis) (Khalil et al., 2022) and neonatal calf diarrhea (Gomez et al., 2017; Weese & Jelinski, 2017).

The most consistent characteristic of microbial communities from diseased animals is a loss of taxonomic diversity (Kostic et al., 2014). Therefore, lower diversity is considered a marker of microbial imbalances in the gastrointestinal tract (Valdes et al., 2018), and it is frequently associated with an increase in potentially pathogenic bacteria and a decrease in health-associated bacteria (Levy et al., 2017). Several studies have investigated dysbiosis in calves and proposed new protocols including fecal microbiota transplantation and or probiotic administration for the prevention and treatment of dysbiosis in place of conventional antibiotic treatments.

Imbalances in the microbial community composition known as dysbiosis are associated to a range of diseases and neonatal calf diarrhea is the main cause of morbidity and mortality in calves during their early life (Malmuthuge & Guan, 2017; Timmerman et al., 2005). Current treatments for diarrhea in calves include fluid therapy, probiotics and antibiotic administration (Henderson et al., 2015; Kim et al., 2014; Malmuthuge et al., 2013). However, the mortality rates remain high, as do the costs of treating sick animals and losses related to performance, especially low weight gain in the months following the diarrhea episode (He et al., 2021; Meale et al., 2016; Millemann, 2009).

The identification of microbial biomarkers is critical for the diagnose of a disease early during infection, however, the identification of reliable biomarkers is often hampered by a low concentration of microbes or biomarkers within host fluids or tissues (Pflughoeft et al., 2019). The identification of microorganisms in feces can be helpful, for example, to identify individual calves at an early stage with an enhance probability to develop dysbiosis/diarrhea, and to take preventive measures before clinical signs occur.

With respect to GIT health in dairy production systems, digestive disorders in lactating dairy cows, such as ruminal acidosis, dominate the scientific literature (Meale et al., 2017). Yet, the calf is the most susceptible animal on the farm with the highest incidence of mortality and morbidity, especially due to diarrhea, compared with the rest of the herd (Malmuthuge & Guan, 2017; Timmerman et al., 2005).

In summary, characterizing the microbiota at the farm should help to fast define and identify causes of dysbiosis and to develop strategies to restore a healthy GIT microbiota. It should also help to predict susceptibility to infection and prevent welfare and health problems since the GIT microbiota composition is involved in the control of pathogen colonization (Gensollen et al., 2016).

1.7 Conclusions

The study of the GIT microbiota of young calves is complex, especially due the quantity of factors that can influence and change microorganisms and their distribution in the GIT. Stress in young calves can contribute to diarrhea and weight loss. The stressors are often animal husbandry practices, including pre-weaning and weaning, vaccination, dehorning, castration, tagging, high temperatures, use of antibiotics, transport, etc.

After birth, the GIT microbiota of calves' and the rumen are not fully developed and functional and stress is often associated with increased risk of diarrhea. Identifying and understanding the colonization and establishment of the young calf microbiota and factors that affect it should help to define and identify dysbiosis and to develop strategies to prevent and treat imbalances restoring a healthy GIT microbiota. Knowing this, host-microbiota interaction should contribute to future studies of the microbiota manipulation and therefore treatment and prevention of diseases associated with dysbiosis.

Chapiter 2 – Scientific manuscript

Dynamics of the fecal microbiota of veal calves after arrival to a rearing unit

Sarah E. I. Crespo[1,2], Sébastien Buczinski[3], Gilles Fecteau[3], Marcio Costa[1]

1. Department of Veterinary Biomedical Sciences, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC J2S 2M2, Canada.
2. Jefo Nutrition Inc., Saint-Hyacinthe, QC J2R 2E7, Canada.
3. Department of Clinical Sciences, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC J2S 2M2, Canada.

Abstract

The microbiota plays an important role in the development of diarrhea in pre-weaned calves. The characterization of the fecal microbiota in health and disease can be critical to unravel the bacterial dynamics associated with diarrhea and antimicrobial usage and help with its prevention and control. The objective of this study was to characterize the dynamics of the fecal microbiota of veal calves entering a rearing unit. Fifty Holstein calves ranging from 8-14 days of life and arriving in a veal unit were enrolled in this study. Fecal samples were collected on arrival and on days 4 ,10 and 24 after arrival. Fecal scores, calves' weight and antibiotic administration were recorded during the study. Sequencing was performed using the Illumina MiSeq platform and data analysed using the software Mothur. Contrary to expectations, richness and diversity were not significant different among healthy and diarrheic calves. The microbiota composition and structure changed among days of collection ($p>0.001$), but the changes were not associated to presence or absence of diarrhea and antibiotic treatment as expected, they are associated to the sampling days. Diarrhea proportion (number of diarrheic calves per day) were numerically higher on days 4, 10 and 24 after arrival. As expected, the relative abundances of bacteria associated to health (i.e., *Bifidobacterium, Lactobacillus and Faecalibacterium)* were decreased in the diarrheic calves. The main limitation of this study is the lack of information about calves' origin before arrival at the rearing unit. This study contributes to the better understanding of the microbial changes related to the stress faced by veal calves and might be the basis for further studies to propose alternative methods of microbiota manipulation to prevent disease and restore health in calves.

Keywords: Fecal microbiota, microbiome, dysbiosis, Calves gastrointestinal microbiota, DNA sequencing, stress.

Introduction

The number of bacterial cells in the human body has been estimated as the same as the number of human cells (Proctor et al., 2013) and this number can rise to approximately 120 times in ruminants (Sender et al., 2016). These microorganisms colonize skin, mouth, airways, vagina, and particularly the gastrointestinal tract (GIT) (Costello et al., 2012; Dominguez-Bello et al., 2010). Ruminants provide the microorganisms with a suitable habitat for growth and the microbes supply protein, vitamins, and short-chain organic acids for the animal (Russell & Rychlik, 2001). The microorganisms in the GIT are also responsible for weight gain, regulate peripheral metabolism, animal performance (Maynard et al., 2012; Sommer & Bäckhed, 2013) and can also influence animal's behavior (Kraimi et al., 2019). Therefore, cattle depend on their gastrointestinal microbiota to digest and convert the plant mass that cannot be directly digested into absorbable nutrients necessary for host health and development (Zhao et al., 2022).

Veal calves receive milk from birth to weaning (approximately 70 days of age depending on the production system) in group pens or more commonly in individual hutch units where calves are fed milk two to three times a day and offered water ad libitum with the goal of transitioning to a solid diet by the age of weaning. The diversity and abundance of GIT bacterial community in calves undergo dynamic shifts during this pre-weaning period (Meale et al., 2016).

During early life and weaning, calves are faced to several sources of stress that include separation of the mother, artificial feeding, transportation and mixing of animals from different farms. These sources of stress promote changes in this gastrointestinal microbiota (Gomez et al., 2017). Calf diarrhea remains the leading cause of mortality in dairy calves (Urie et al., 2020) therefore, a properly functioning intestinal microbiota is critical for maintenance of health and changes in the normal fecal microbiota composition (dysbiosis) are related to the occurrence intensity and the duration of diarrhea (Gomez et al., 2017).

In calves, the use of antibiotics to prevent diarrhea and respiratory diseases is common (Gomez et al., 2017). Despite the benefits observed with the use of antibiotics as growth promoters in the animal production system, there is an increasing pressure for this practice to be prohibited,

mainly due to the possibility of selection of antibiotic-resistant bacteria, which can contributes to resistant infections in humans (Weese et al., 2015).

The search for alternatives to the use of antibiotics in animal feeding is a growing concern, and a better understanding of the dynamics of the microbial community is essential to understand the changes that occur in the GIT microbiota during the early stage of the calf's life is essential. However, limited knowledge is available for the early life GIT microbiota and its relationship with the calf's performance. Recent research highlights microbiota as an important factor in the immune function development and in the maintenance of the neonate GIT health, making the manipulation of the GIT microbiota a potential source to improve health and by reducing the prevalence of diarrhea and other neonatal diseases (Gensollen et al., 2016; Gomez et al., 2019). Thus, a better understanding of the structure of the gastrointestinal microbiota is instrumental in both production and scientific inquiry before any manipulation.

Dairy and beef industries are major commodity sectors in the world's economy and improvements in animal production are necessary to improve animal welfare and a sustainable production system. This study aimed to investigate the longitudinal changes of the gastrointestinal microbiota of veal calves after arrival in an operational unit. We hypothesized that the calves' fecal microbiota would change after arrival and that calves with diarrhea would have a different microbiota when compared with non-diarrheic calves.

Materials and Methods

Animals and Sample Collection

This trial was conducted at one commercial veal farm in Quebec (Saint-Liboire, QC, Canada). Fifty Holstein calves, both sexes, ranging from 8 to 14 days of age were enrolled in this study. The animals came from different farms and were purchased at an action 20 min far from the rearing farm. Information about the preview origin of the calves was not available.

Fecal samples were collected when arriving at the veal farm (D0), and at days 4 (D4), 10 (D10) and 24 (D24) after arrival. Samples were taken directly from the rectum by use of a glove when defecation was not spontaneous. A minimum of 3 - 5g of feces per sampling was taken and placed

in a plastic bag (Whirl-Pak® Write On Bags, 1 oz, Madison, WI, USA). Fecal scores were evaluated by the same experienced veterinarian and recorded based on a 0 to 3 system: 0=normal consistency; 1=semi formed or pasty; 2=loose feces and 3=watery, using a calf health scoring guide by the University of Wisconsin-Madison School of Veterinary Medicine. A diarrheic calf was defined as an animal with a fecal score of 2 or 3. After sampling, feces were refrigerated and transported immediately and then stored at −80◦C for further analysis.

All animals had *ad libitum* access to water and were fed with 2 L of milk replacer (Goliath XLR 27–16, La Coop, Montreal, QC, Canada) in buckets three times per day. During this study, calves received only milk and remained individually housed in pens made of hardwood boards. All pens were in the same calf house and animals were randomly allocated. The calf house was equipped with controlled ventilation and temperature, air humidity and ammonia concentration. Animals were weighed at the action and before exiting to slaughter. The body weight was determined using an electronic scale on both occasions.

Ethics statement

This study was approved by the University of Montreal – Faculté de Médecine vétérinaire - Animal Care Committee (22-Rech-2178).

DNA extraction

Total DNA was extracted from feces using the DNeasy PowerSoil PRO kit (QIAGEN, Toronto, ON, Canada) in accordance with the manufacturer's instructions. DNA concentrations were estimated using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE) at wavelengths of 230, 260, and 280 nm.

16S rRNA gene amplicon sequencing

Total extracted DNA was amplified with a set of oligonucleotide primers targeting the V4 region of the 16 rRNA gene using the primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) with overhanging adapters for annealing to Illumina universal index sequencing adaptors (Klindworth et al., 2013). Sequencing was performed using an Illumina MiSeq platform for 250 cycles from each end at the Génome Québec Innovation Centre.

Bioinformatic analysis

Bioinformatic analysis was performed using the software Mothur (v1.44.2) (Schloss et al. 2009) following the Standard Operating Procedure previously described (Kozich et al., 2013). Sequencing reads were aligned with the SILVA reference database, clustered at 97% similarity and classified using the Ribosomal Databank Project (RDP). Sequences classified as the same genus (94% similarity) were clustered together for further analyses (Phylotypes).

The Chao richness estimator, Simpson's diversity index, and Shannon index were calculated for characterization of richness and diversity. Those indices were compared between animals at the different sampling times using one-way analysis of variance (ANOVA). Beta-diversity was characterized by the Jaccard and Yue and Clayton indexes to evaluate community membership and structure, respectively. A 2-dimensional Principal Coordinate Analysis (PCoA) plot was generated to visualize the similarity between samples. Analysis of molecular variance (AMOVA) was used to determine significance of clustering between samples at different sampling times. The most abundant bacteria (>1%) were visualized by generating bar charts representing the relative abundance of the main phyla and genera found. The linear discriminant analysis effect size (LEfSe) was used to detect significant differences in relative abundances over time (Segata et al., 2011).

Average weight of animals that had diarrhea or not during the study was determined using Analyze de covariance (ANCOVA). Initial weight was used as covariant.

Diarrhea incidence was calculated using ANOVA with Tukey test with 95% CI with a statistical difference if $p < .05$.

Results

Microbiota analysis

A total of 6,248,512 reads were obtained of which 5,035,713 were high quality sequences that therefore were used for final analysis. One sample from day 4 (D4_8520) did not yield an adequate number of reads and was excluded of the analyses.

The proportion of calves with diarrhea and treated with antimicrobials increased, especially after D4 (D0-24% of the calves presented diarrhea; D4-48%; D10-34% and D24-39%) (p >0.09). All the calves that had diarrhea received treatment with antibiotics. The most used antibiotic was Spectinomycin-lincomycin IM (8ml/50kg – BID in the first day of diarrhea and SID for the next four days). Only one calf died on D4 of undetermined cause.

Incidence of diarrhea was measured during the sampling days and was not statistically different between sampling dates (D0 vs. D4, D10 and D24; p=0.09) (Figure 1).

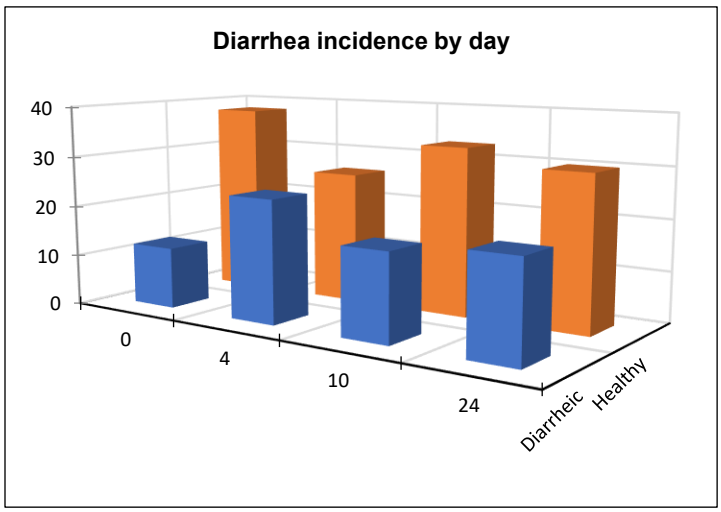


Figure 1. – Diarrhea incidence according to sampling days (D0, D4, D10 and D24 – AMOVA – Tuckey test).

In addition, animals were weighted at the auction exit and just before slaughter. Weigh was compared among animals taking in consideration diarrhea (Figure 2) and results are not statistically different.

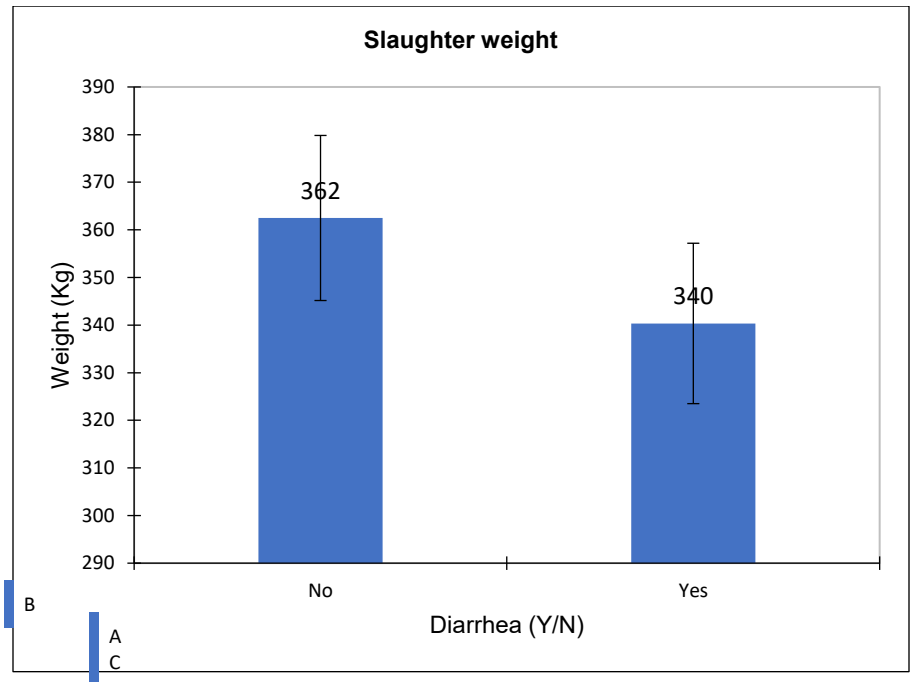


Figure 2. – Comparation of the average weight (kg) of animals that had diarrhea or not during the study. Initial weight (auction exit) was used as a covariant (ANCOVA - Analyze de covariance; p=0.072).

Alpha Diversity

A significant difference in richness (Chao) and diversity (Inv. Simpson and Shannon index) was observed among animals over time (Figure 3) but not among health status (p > 0.1).

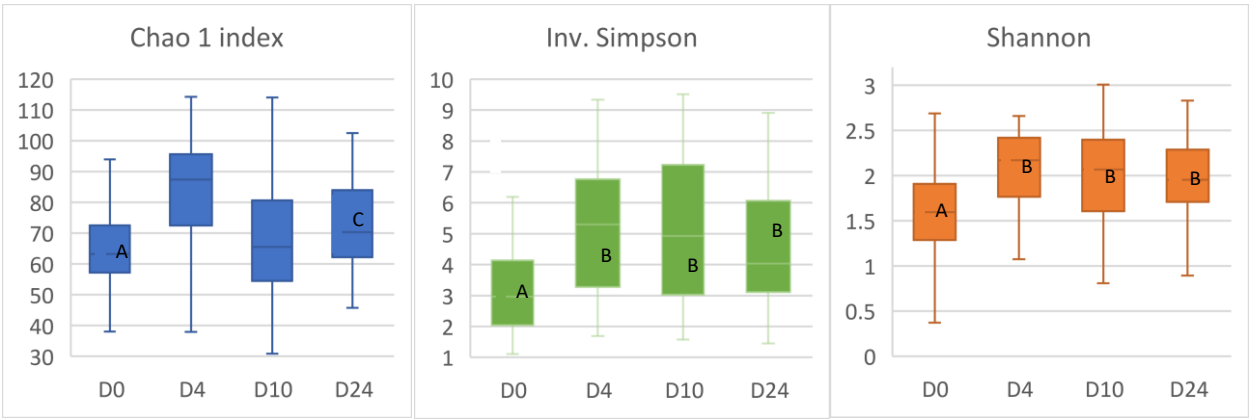
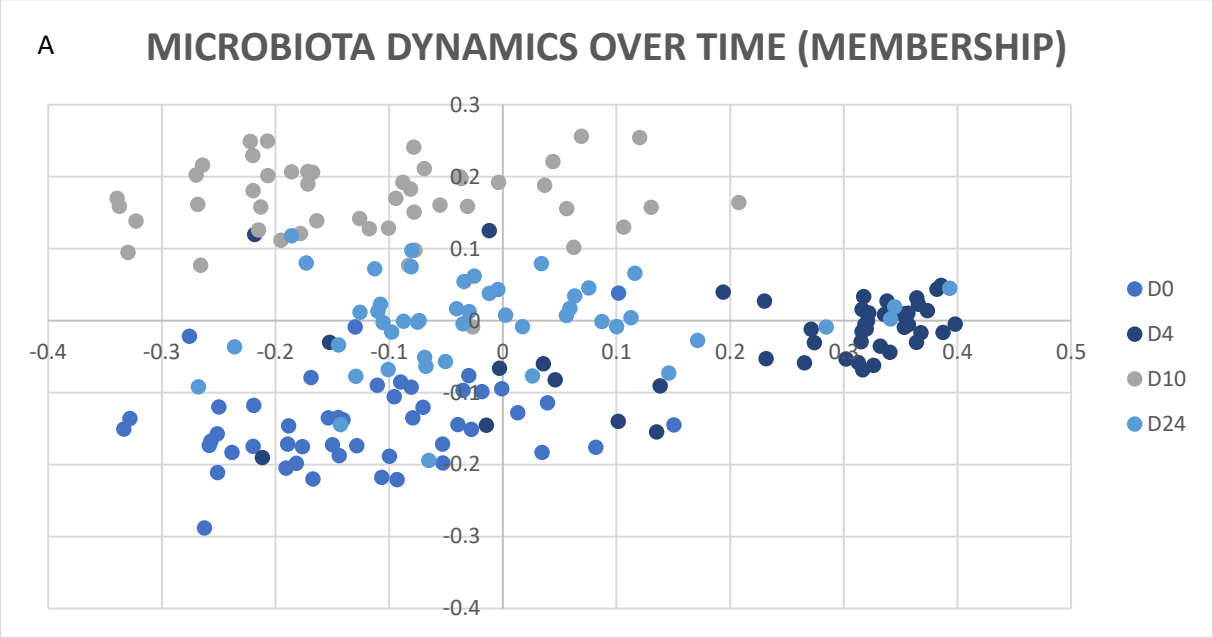


Figure 3. – Chao richness estimator, Simpson's diversity index, and Shannon index for feces on each one of 4 sampling days (D0, D4, D10 and D24). Different letters indicate significant

differences (Chao1 $p < 0.001$; Inv. Simpson $p < 0.01$ and Shannon, $p < 0,001$). Bars represent means.

Principal Coordinate Analysis (PCoA)

The microbiota dynamics over time can be observed above for both membership and structure (Figure 4A and B). Significant differences were observed in beta diversity membership (Figure 5A, $p < 0.001$) (Figure 5B, $p < 0.001$) and structure during the studied days, but not among health status (healthy or diarrheic).



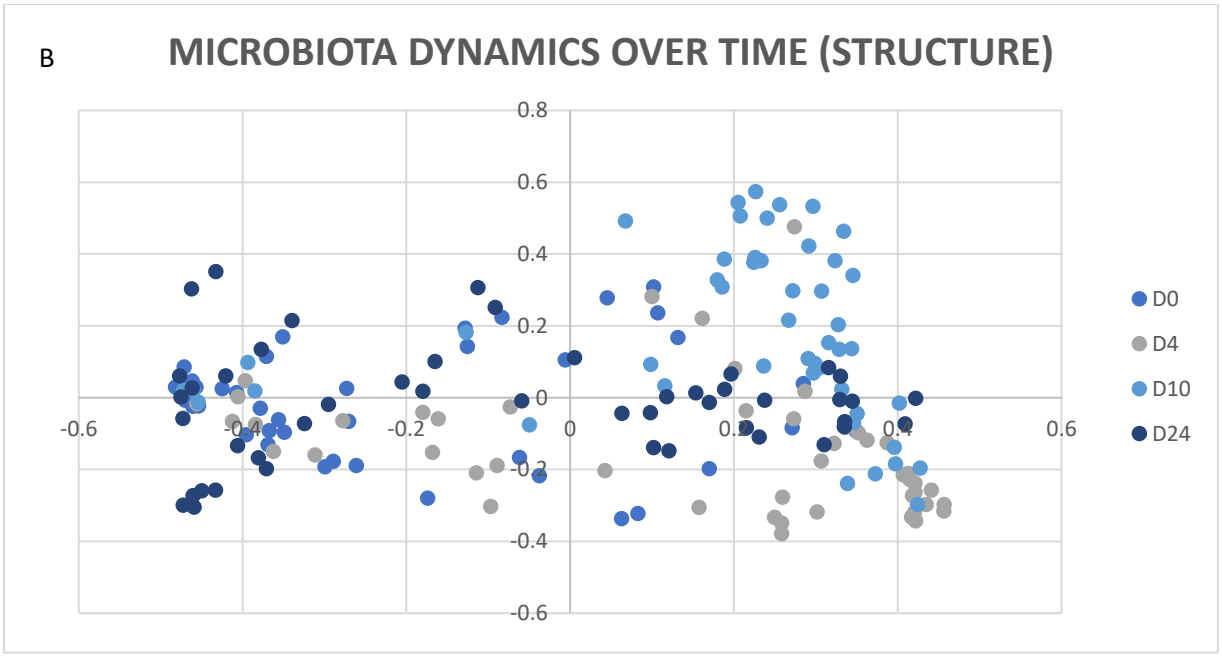
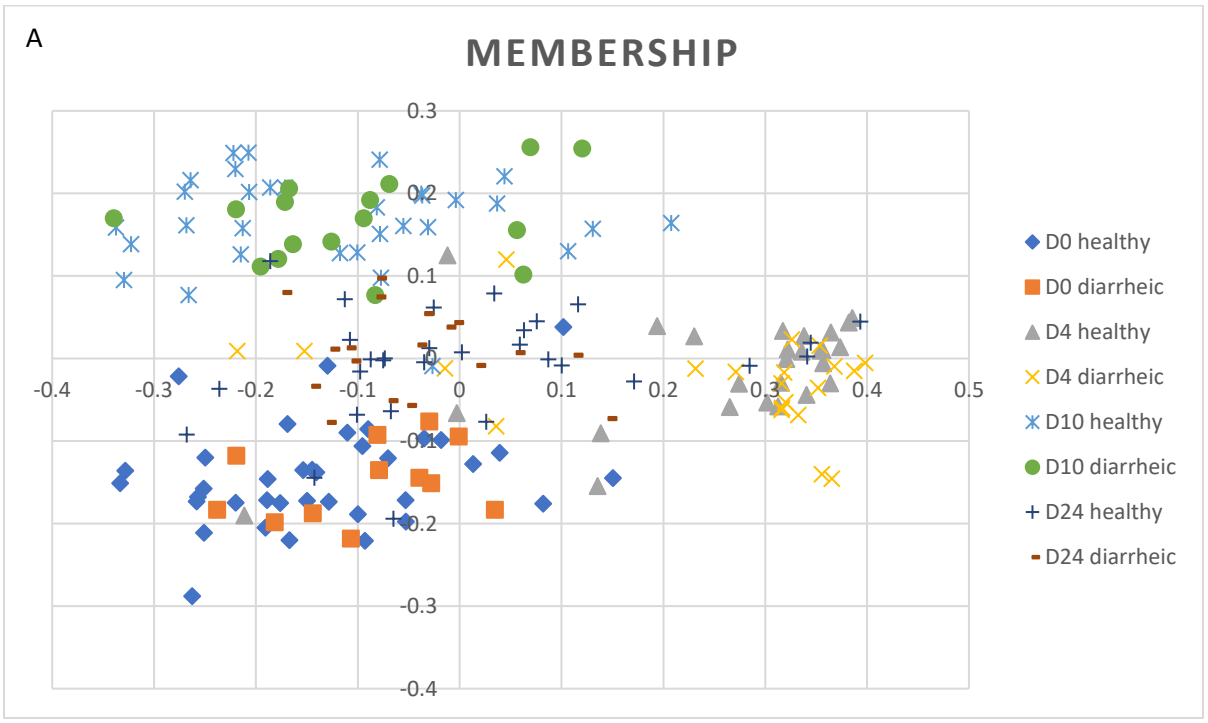


Figure 4. – Principal coordinate analysis (PCoA) of bacterial communities' membership showing the microbiota dynamics over time.



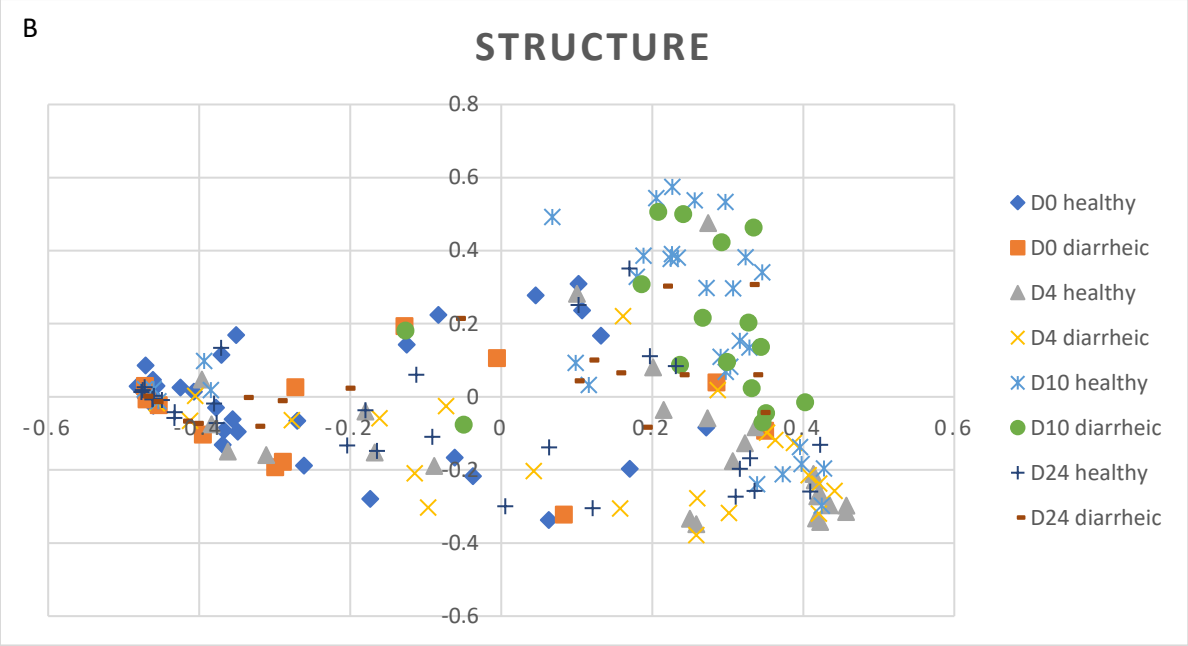


Figure 5. – Principal coordinate analysis (PCoA) of bacterial communities' membership of healthy and diarrheic calves (A) and structure of healthy and diarrheic (B) calves present in feces on days D0, D4, D10 and D24.

Relative Abundances

The relative abundances at the phylum level found in feces are shown in Figure 6. The most abundant taxa at the phylum level in the feces were Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria.

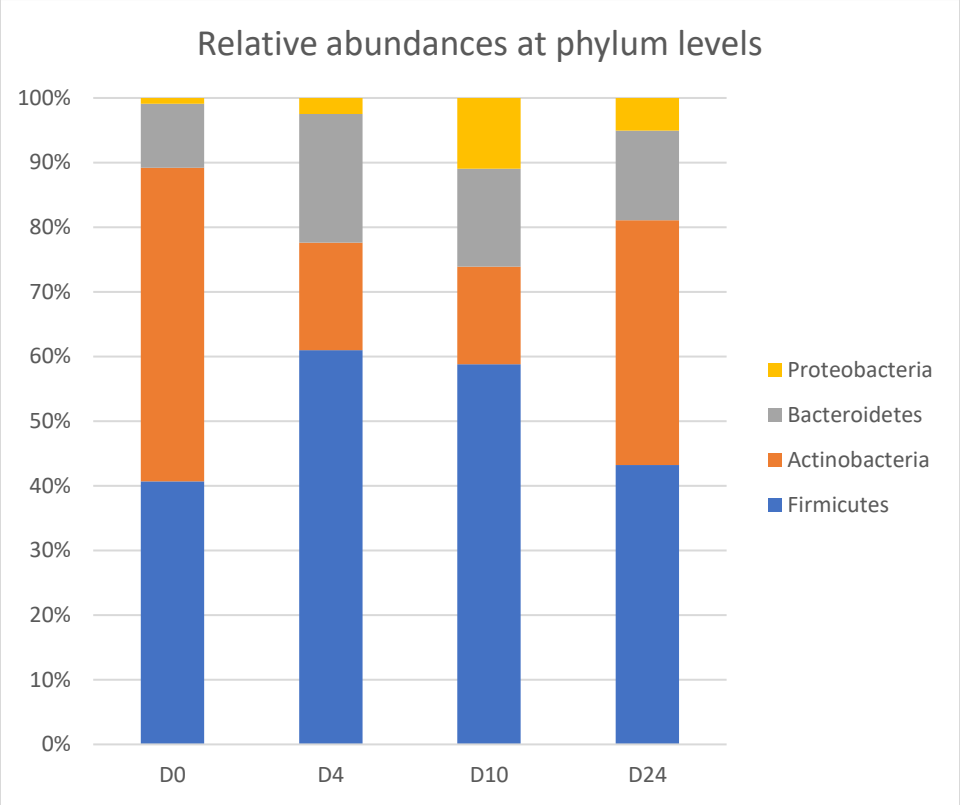


Figure 6. – Relative abundances of the main taxa (1% abundance) present in the calves' feces according to the days of sampling (0, 4 10 and 24), at phylum level.

The relative abundances at genus level found in feces are shown in Figure 7. The most abundant taxa were *Bifidobacterium*, unclassified *Lachnospiraceae*, *Subdoligranulum*, *Phocaeicola*, *Lactobacillus*, *Collinsella*, *Butyricicoccus*, *Escherichia/Shiguela*, *Prevotella*, *Bacteroides*, *Megamonas*, *Faecalibacterium*, *Blautia*, unclassified *Ruminococcaceae*, *Mediterraneibacter*, Limosilactobacillus and Veillonella.

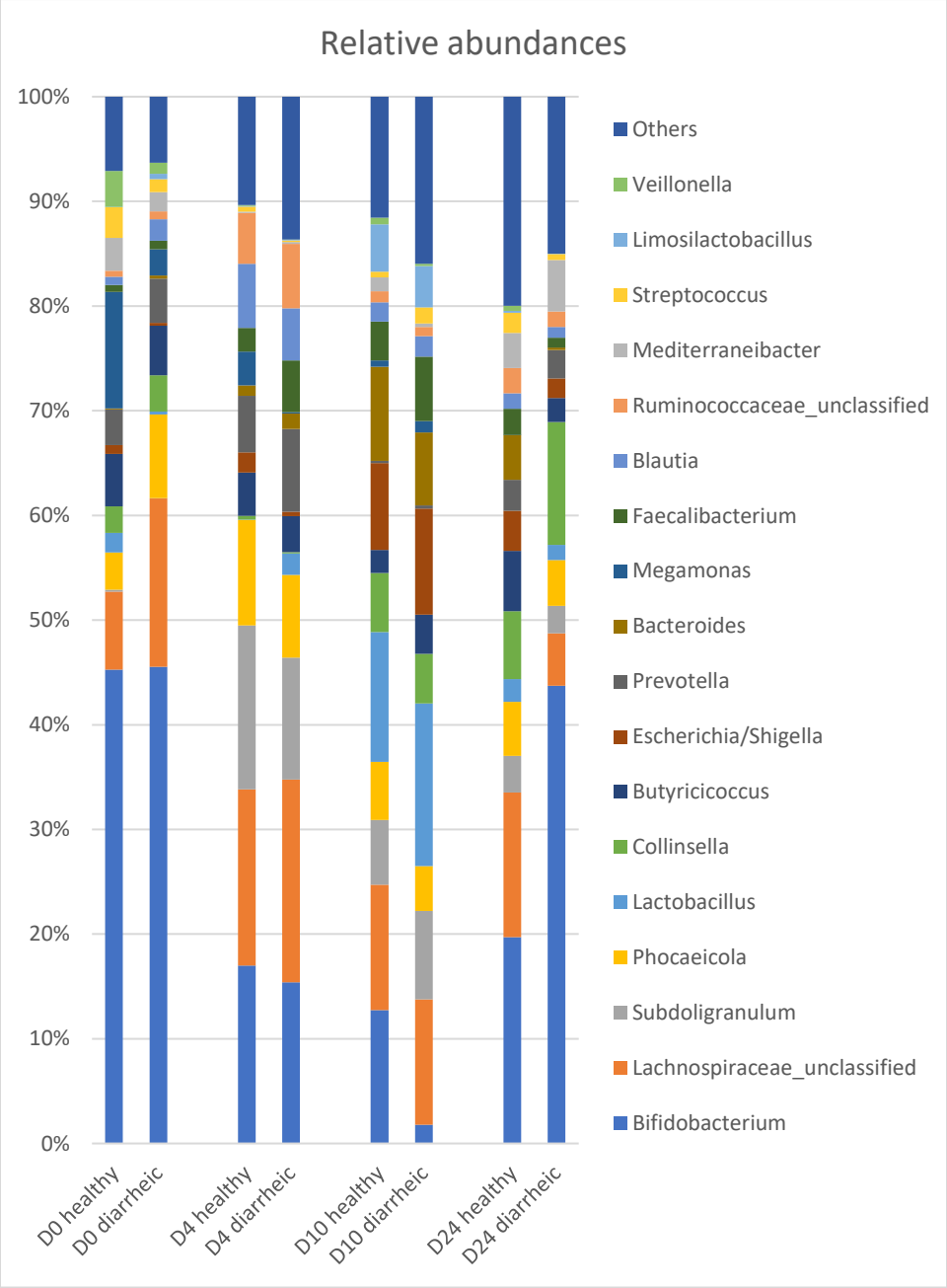


Figure 7. – Relative abundances of predominant bacteria present in the calves' feces according to the days of sampling (0, 4 10 and 24) and healthy status (healthy or diarrheic), at genus level. The eighteen most abundant genera are represented.

Linear discriminant analyses (LDA) Effect Size (LEfSe)

To further evaluate the changes in fecal microbiota associated with sampling days, the differences in relative abundances between calves were compared using the LEfSe algorithm (log score threshold ≥ 3) (Figure 8).

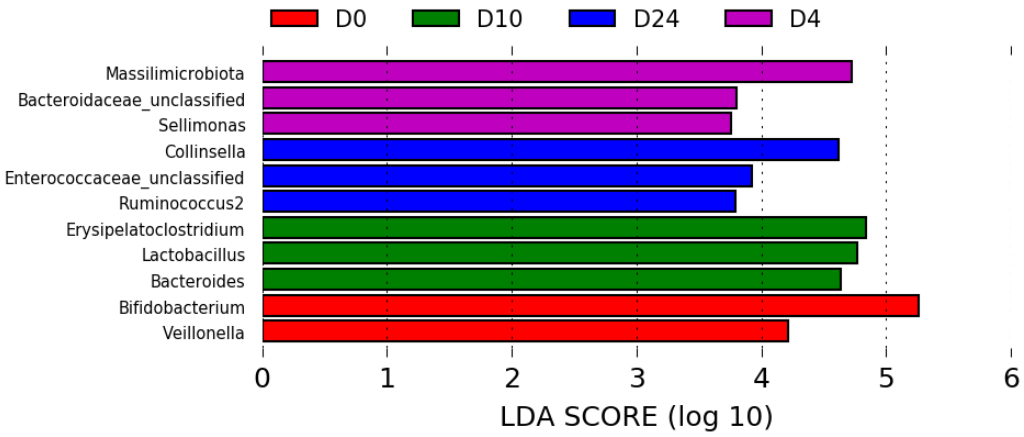


Figure 8. – Lefse Predicted functional composition of metagenomes based on 16S rRNA gene sequencing data revealed differentially enriched bacterial functions associated to sampling days.

Discussion

This study characterized the fecal microbiota of young veal calves from different farms arriving at a rearing unit, using high throughput DNA sequencing. The faecal bacterial community of the veal calves was dominated by potential lactose- and starch-degrading bacteria. Although it is difficult to define normal calf microbiota, general trends can be inferred from previous studies. A higher abundance of Bifidobacterium found on D0 is expected for milk-consuming calves. These bacteria were found to have several beneficial effects on host health, such as pathogen defense, and modulation of immune and inflammatory processes (Du et al., 2023; Oikonomou et al., 2013). In addition, *Bacteroides* and *Faecalibacterium* have been linked with lower disease susceptibility in calves (Oikonomou et al., 2013; Zeineldin et al., 2018). *Lactobacillus,* for example, has been shown to mitigate *Escherichia coli-Shigella* presence, reinstating a balanced fecal microbiota (Dong et al., 2021). It also plays a crucial role in maintaining intestinal health, which contributes to a lower pH

in the intestinal tract, which inhibits the growth of harmful pathogens (Dempsey & Corr, 2022). Therefore, having high levels of *Lactobacillus* is considered a key indicator of a healthy gut environment.

We speculated that the rapid decreasing in the relative abundances of those health-related taxa in our study is due to the stress experienced by the animals that include marketing, diet changing, transportation, etc. Rapid changes in fecal microbiota occur during the first 48 hours of diarrhea, and the time from diarrhea onset to sampling appears to affect the bacterial composition present in the feces of the sick animals (Li et al., 2023). A recent study evaluated the effect of time of sample collection after onset of diarrhea on fecal microbiota composition of calves and found that bacterial membership and structure were significantly different between the diarrhea episode and 24h and diarrhea episode and 24-48h (Li et al., 2023)

In addition, a recent study evaluating the effect of cattle source (farm-direct or auction market-derived) and rest stop duration (0 or 8 h of rest) on the upper respiratory tract microbiota of young calves and showed that the bacterial community structure is altered by feedlot placement. They concluded that the transportation and the auction market placement may be risk factors for respiratory diseases (Uddin et al., 2023).

Differences in the fecal microbiome composition across diarrheic and non-diarrheic calves have been previously reported (Chen et al., 2022; Holman et al., 2019; Obregon-Gutierrez et al., 2022), and differences in the microbiome profile of calves with and without diarrhea are expected given that diarrhea is a complex disorder often associated with dysbiosis regardless of specific pathogens (Alipour et al., 2018; Gomez et al., 2017). An increased diversity has been associated with lower incidence of diarrhea in healthy calves and increased weight gain (Malmuthuge & Griebel, 2019; Oikonomou et al., 2013). Interestingly in this study, the alpha diversity was higher on days 4, 10 and 24 after arrival, same days with increased incidence of diarrhea among calves.

Interestingly, in the PCoA plots generated, animals clustered according to the sampling day, regardless of health status (healthy or diarrheic). Therefore, in this study there were significant differences in microbiota structure and membership among days, but not among health status (healthy and diarrheic calves). This observation was surprising given the differences among

healthy and diarrheic calves are expected and have been preview published (Dias et al., 2018; Garcia Dias & Ametaj, 2017; Mao et al., 2015). These results highlight the need to look beyond simple comparison of relative abundances when trying to interpret the microbiota, because relative abundance changes can be influenced by several factors such as use of antibiotics and diarrhea duration.

Calf diarrhea also provokes the overuse of antibiotics. Antibiotics have been widely used to treat or prevent diarrhea and promote growth in calves (Constable, 2004; Du et al., 2023; Holman et al., 2019), however, accumulating evidence have shown that the use of antibiotics in animal husbandry is associated with many adverse effects. The long terms effects of antibiotics on the GIT microbiota and consequently calves' health and performance are not completely understood. The weight of the animals that had diarrhea and received antibiotics was lower, but not statistically different from non-diarrheic animals.

The main limitations of this study are the lack of previous information about the calves before arrival at the rearing unit, such as the origin of the animals, type of feeding, type of facilities, distance of the farm from the auction market and time and type of transportation. Our findings may contribute to better understanding the GIT microbial dynamics during the preweaning stage and to developing strategies of microbiota manipulation to improve calf health.

Conclusions

The current study demonstrated that the GIT microbiota of young calves rapidly changes in the first days after arrival in the rearing unit. The factors related to these changes are likely related to the stress this category of animals are exposed. This study contributes to further investigations addressing the impact the GIT microbiota in the calves' health.

Funding

This study was supported by Probiotech and Mitacs.

Acknowledgements

We would like to acknowledge Délimax for giving us access to their facilities during the experimental process.

Conflicts of interest

The authors declare no conflicts of interest.

References

Alipour, M. J., Jalanka, J., Pessa-Morikawa, T., Kokkonen, T., Satokari, R., Hynönen, U., Iivanainen, A., & Niku, M. (2018). The composition of the perinatal intestinal microbiota in cattle. *Scientific Reports*, *8*(10437), 1–14. https://doi.org/10.1038/s41598-018-28733-y

AlShawaqfeh, M. K., Wajid, B., Minamoto, Y., Markel, M., Lidbury, J. A., Steiner, J. M., Serpedin, E., & Suchodolski, J. S. (2017). A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbio, M. K., Wajid, B., Minamoto, Y., Markel, M., Lidbury, J. A., Steiner, J. M., Serpedin, E., & Suchodolski, J. S. (2017). A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiology Ecology*, *93*(11), 1–8. https://doi.org/10.1093/femsec/fix136

Amin, N., & Seifert, J. (2021). Dynamic progression of the calf ' s microbiome and its influence on host health. *Computational and Structural Biotechnology Journal*, *19*, 989–1001. https://doi.org/10.1016/j.csbj.2021.01.035

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., Versalovic, J., Young, V., & Finlay, B. B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host and Microbe*, *12*(5), 611–622. https://doi.org/10.1016/j.chom.2012.10.012

Bager, P., Wohlfahrt, J., & Westergaard, T. (2008). Caesarean delivery and risk of atopy and allergic disesase: Meta-analyses. *Clinical and Experimental Allergy*, *38*(4), 634–642. https://doi.org/10.1111/j.1365-2222.2008.02939.x

Barko, P. C., McMichael, M. A., Swanson, K. S., & Williams, D. A. (2018). The Gastrointestinal Microbiome: A Review. *Journal of Veterinary Internal Medicine*, *32*(1), 9–25. https://doi.org/10.1111/jvim.14875

Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Bignell, H. R., & Boutell, J. M. (2008). An improved ant colony algorithm used for unscented Kalman filter. *Accurate Whole*

Human Genome Sequencing Using Reversible Terminator Chemistry, *6*(456 (7218)), 53–59. https://doi.org/10.1038/nature07517.Accurate

Cani, P. D., Van Hul, M., Lefort, C., Depommier, C., Rastelli, M., & Everard, A. (2019). Microbial regulation of organismal energy homeostasis. *Nature Metabolism*, *1*(1), 34–46. https://doi.org/10.1038/s42255-018-0017-4

Chen, H., Liu, Y., Huang, K., Yang, B., Zhang, Y., Yu, Z., & Wang, J. (2022). Fecal microbiota dynamics and its relationship to diarrhea and health in dairy calves. *Journal of Animal Science and Biotechnology*, *13*(1), 1–20. https://doi.org/10.1186/s40104-022-00758-4

Choudhury, P. H., Salem, A. Z. M., Jena, R., Kumar, S., Singh, R., & Puniya, A. kumar. (2015). Rumen microbiology: From evolution to revolution. In *Rumen Microbiology: From Evolution to Revolution*. https://doi.org/10.1007/978-81-322-2401-3

Constable, P. D. (2004). Antimicrobial Use in the Treatment of Calf Diarrhea Change in Small Intestinal Bacterial Flora in Calves with Diarrhea. *J Vet Intern Med*, *18*, 8–17.

Costa, M. C., & Weese, J. S. (2019). Methods and basic concepts for microbiota assessment. *Veterinary Journal*, *249*, 10–15. https://doi.org/10.1016/j.tvjl.2019.05.005

Costa, M., Stämpfli, H. R., Allen-Vercoe, E., & Weese, J. S. (2015). Development of the faecal microbiota in foals. *Equine Veterinary Journal*, *0*, 1–8. https://doi.org/10.1111/evj.12532

Costa, M., & Weese, J. S. (2019). Methods and basic concepts for microbiota assessment. *Veterinary Journal*, *249*, 10–15. https://doi.org/10.1016/j.tvjl.2019.05.005

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J. M., & Relman, D. A. (2012). The application of ecological theory towrds an understanding of the human microbiome. *Science*, *336*(6086), 1255–1262. https://doi.org/10.1126/science.1224203.The

de Tejada, B. M. (2014). Antibiotic use and misuse during pregnancy and delivery: Benefits and risks. *International Journal of Environmental Research and Public Health*, *11*(8), 7993–8009. https://doi.org/10.3390/ijerph110807993

Dempsey, E., & Corr, S. C. (2022). Lactobacillus spp. for Gastrointestinal Health: Current and

Future Perspectives. *Frontiers in Immunology*, *13*(March 2020), 1–15. https://doi.org/10.3389/fimmu.2022.840245

Diao, Q., Zhang, R., & Fu, T. (2019). *Review of Strategies to Promote Rumen Development in Calves*. 1–15.

Dias, J., Marcondes, I., Souza, M. De, Noronha, M. F., Resende, T., Machado, S., & Mantovani, C. (2018). Bacterial Community Dynamics across the Gastrointestinal Tracts of Dairy Calves during Preweaning Development. *Applied and Environmental Microbiology*, *84*(9), 1–12.

Dill-Mcfarland, K. A., Breaker, J. D., & Suen, G. (2017a). Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation. *Scientific Reports*, *7*(June 2016), 1–12. https://doi.org/10.1038/srep40864

Dill-Mcfarland, K. A., Breaker, J. D., & Suen, G. (2017b). Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation. *Scientific Reports*, *7*(January), 1–12. https://doi.org/10.1038/srep40864

Dill-McFarland, K. A., Weimer, P. J., Breaker, J. D., & Suen, G. (2019). Diet Influences Early Microbiota Development in Dairy Calves without Long-Term Impacts on Milk Production. *Applied and Environmental Microbiology*, *85*(2), 1–12. https://doi.org/10.1128/AEM.02141-18

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107

Dong, H., Liu, B., Li, A., Iqbal, M., Mehmood, K., Jamil, T., Chang, Y. F., Zhang, H., & Wu, Q. (2021). Microbiome Analysis Reveals the Attenuation Effect of Lactobacillus From Yaks on Diarrhea via Modulation of Gut Microbiota. *Frontiers in Cellular and Infection Microbiology*, *10*(February), 1–13. https://doi.org/10.3389/fcimb.2020.610781

Du, W., Wang, X., Hu, M., Hou, J., Du, Y., Si, W., Yang, L., Xu, L., & Xu, Q. (2023). Modulating

gastrointestinal microbiota to alleviate diarrhea in calves. *Frontiers in Microbiology*, *14*(June), 1–13. https://doi.org/10.3389/fmicb.2023.1181545

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., & Relman, D. A. (2005). Diversity of the Human Intestinal Microbial Flora. *Science*, *308*(5728), 1635–1638. https://doi.org/10.1126/science.1110591

Eisler, M. C., Lee, M. R. F., Tarlton, J. F., & Martin, G. B. (2014). Steps to sustainable livestock. *Nature*, *507*(7490), 32–34. https://doi.org/10.1038/507032a

Fan, P., Bian, B., Teng, L., Nelson, C. D., Driver, J., Elzo, M. A., & Jeong, K. C. (2020). Host genetic effects upon the early gut microbiota in a bovine model with graduated spectrum of genetic variation. *ISME Journal*, *14*(1), 302–317. https://doi.org/10.1038/s41396-019-0529-2

Garcia Dias, A. L., & Ametaj, B. N. (2017). Cattle Gastrointestinal Tract Microbiota in Health and Disease. In *Periparturient Diseases of Dairy Cows: A Systems Biology Approach* (Vol. 1, pp. 71–91). https://doi.org/10.1007/978-3-319-43033-1

Gensollen, T., Iyer, S. S., Kasper, D. L., Blumberg, R. S., & Medical, H. (2016). How colonization by microbiota in early life shapes the immune 1. Gensollen T, Iyer SS, Kasper DL, Blumberg RS, Medical H. How colonization by microbiota in early life shapes the immune system. Science. 2016;352(6285):539-544. doi:10.1126/science.aad9378. *Science (New York, N.Y.)*, *352*(6285), 539–544. https://doi.org/10.1126/science.aad9378.How

Gibbons, J. F., Boland, F., Buckley, J. F., Butler, F., Egan, J., Fanning, S., Markey, B. K., & Leonard, F. C. (2013). *Influences on antimicrobial prescribing behaviour of veterinary practitioners in cattle practice in Ireland*. https://doi.org/10.1136/vr.100782

Gomez, D. E., Arroyo, L. G., Costa, M. C., Viel, L., & Weese, J. S. (2017). Characterization of the Fecal Bacterial Microbiota of Healthy and Diarrheic Dairy Calves. *Journal of Veterinary Internal Medicine*, *31*(3), 928–939. https://doi.org/10.1111/jvim.14695

Gomez, D. E., Galvão, K. N., Rodriguez-Lecompte, J. C., & Costa, M. C. (2019). The Cattle Microbiota and the Immune System: An Evolving Field. *Veterinary Clinics of North America - Food Animal Practice*, *35*(3), 485–505. https://doi.org/10.1016/j.cvfa.2019.08.002

Gomez, D. E., Li, L., Goetz, H., Macnicol, J., Gamsjaeger, L., & Renaud, D. L. (2022). *Calf Diarrhea Is Associated With a Shift From Obligated to Facultative Anaerobes and Expansion of Lactate-Producing Bacteria*. *9*(March), 1–8. https://doi.org/10.3389/fvets.2022.846383

Govil, K., Yadav, D. S., Patil, A. K., Nayak, S., Baghel, R. P. S., & Yadav, P. K. (2017). *Feeding management for early rumen development in calves*. *5*(3), 1132–1139.

Hang, B. P. T., Wredle, E., & Dicksved, J. (2021). Analysis of the developing gut microbiota in young dairy calves—impact of colostrum microbiota and gut disturbances. *Tropical Animal Health and Production*, *53*(1). https://doi.org/10.1007/s11250-020-02535-9

Hartinger, T., Pacífico, C., Poier, G., Terler, G., & Klevenhusen, F. (2022). Shift of dietary carbohydrate source from milk to various solid feeds reshapes the rumen and fecal microbiome in calves. *Scientific Reports*, 1–12. https://doi.org/10.1038/s41598-022-16052-2

Hasan, N., & Yang, H. (2019). Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ*, *2019*(8), 1–31. https://doi.org/10.7717/peerj.7502

He, Y., Maltecca, C., & Tiezzi, F. (2021). Potential use of gut microbiota composition as a biomarker of heat stress in monogastric species: A review. *Animals*, *11*(6). https://doi.org/10.3390/ani11061833

Heinrichs, J. (2005). *Rumen Development in the Dairy Calf Rudimentary Reticulo-Rumen Changes in Rumen Epithelium*. *17*, 179–187.

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., & Janssen, P. H. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, *5*(14567), 1–13. https://doi.org/10.1038/srep14567

Holman, D. B., Yang, W., & Alexander, T. W. (2019). Antibiotic treatment in feedlot cattle: A longitudinal study of the effect of oxytetracycline and tulathromycin on the fecal and nasopharyngeal microbiota. *Microbiome*, *7*(1), 1–14. https://doi.org/10.1186/s40168-019-0696-4

Holmes, A. H., Moore, L. S. P., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. V. (2015). *Antimicrobials : access and sustainable eff ectiveness 2 Understanding the mechanisms and drivers of antimicrobial resistance*. *6736*(15). https://doi.org/10.1016/S0140-6736(15)00473-0

Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjölund-Karlsson, M., Jansson, J. K., & Engstrand, L. (2010). Short-term antibiotic treatment has differing long- term impacts on the human throat and gut microbiome. *PLoS ONE*, *5*(3). https://doi.org/10.1371/journal.pone.0009836

Jami, E., Israel, A., Kotser, A., & Mizrahi, I. (2013). Exploring the bovine rumen bacterial community from birth to adulthood. *International Society for Microbial Ecology*, *7*(6), 1069–1079. https://doi.org/10.1038/ismej.2013.2

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., & Sasikala, M. (2015). *role of the normal gut microbiota*. *21*(29), 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787

Kamada, N., Chen, G. Y., Inohara, N., & Núñez, G. (2013). *Control of pathogens and pathobionts by the gut microbiota*. *14*(7), 685–690. https://doi.org/10.1038/ni.2608

Kamra, D. N. (2005). Rumen Microbial Ecosystem. *Current Science*, *89*(1), 124–135. https://doi.org/10.1146/annurev.es.06.110175.000351

Kelly, D., King, T., & Aminov, R. (2007). Importance of microbial colonization of the gut in early life to the development of immunity. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *622*(1–2), 58–69. https://doi.org/10.1016/j.mrfmmm.2007.03.011

Khalil, A., Batool, A., & Arif, S. (2022). Healthy Cattle Microbiome and Dysbiosis in Diseased Phenotypes. *Ruminants*, *2*(1), 134–156. https://doi.org/10.3390/ruminants2010009

Kim, M., Kim, J., Kuehn, L. A., Bono, J. L., Berry, E. D., Kalchayanand, N., Freetly, H. C., Benson, A. K., & Wells, J. E. (2014). Investigation of bacterial diversity in the feces of cattle fed different diets. *Journal of Animal Science*, *92*(2), 683–694. https://doi.org/10.2527/jas2013-6841

Klein-Jöbstl, D., Iwersen, M., & Drillich, M. (2014). Farm characteristics and calf management

practices on dairy farms with and without diarrhea: A case-control study to investigate risk factors for calf diarrhea. *Journal of Dairy Science*, *97*(8), 5110–5119. https://doi.org/10.3168/jds.2013-7695

Klein-Jöbstl, D., Quijada, N. M., Dzieciol, M., Feldbacher, B., Wagner, M., Drillich, M., Schmitz-Esser, S., & Mann, E. (2019). Microbiota of newborn calves and their mothers reveals possible transfer routes for newborn calves' gastrointestinal microbiota. *Plos One*, *14*(8), e0220554. https://doi.org/10.1371/journal.pone.0220554

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), 1–11. https://doi.org/10.1093/nar/gks808

Kolbert, C. P., & Persing, D. H. (1999). Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Current Opinion in Microbiology*, *2*(3), 299–305. https://doi.org/10.1016/S1369-5274(99)80052-6

Kostic, A. D., Ramink, X. J., & Dirk, G. (2014). The Microbiome in Inflammatory Bowel Diseases: Current Status and the Future Ahead. *Gastroenterology*, *146*(6), 1489–1499. https://doi.org/10.1053/j.gastro.2014.02.009.The

Kraimi, N., Dawkins, M., Gebhardt-Henrich, S. G., Velge, P., Rychlik, I., Volf, J., Creach, P., Smith, A., Colles, F., & Leterrier, C. (2019). Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: A review. *Physiology & Behavior*, *210*(112658), 1–12. https://doi.org/10.1016/j.physbeh.2019.112658

Kuo, C. H., Kuo, H. F., Huang, C. H., Yang, S. N., Lee, M. S., & Hung, C. H. (2013). Early life exposure to antibiotics and the risk of childhood allergic diseases: An update from the perspective of the hygiene hypothesis. *Journal of Microbiology, Immunology and Infection*, *46*(5), 320–329. https://doi.org/10.1016/j.jmii.2013.04.005

Larsen, O. F. A., & Claassen, E. (2018). The mechanistic link between health and gut microbiota diversity. *Scientific Reports*, *8*(1), 6–10. https://doi.org/10.1038/s41598-018-20141-6

Leeming, E. R., Johnson, A. J., Spector, T. D., & Roy, C. I. Le. (2019). Effect of Diet on the Gut Microbiota: Rethinking Intervention Duration. *Nutrients*, *11*(2862), 1–28.

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. *Nature Reviews Immunology*, *17*(4), 219–232. https://doi.org/10.1038/nri.2017.7

Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., & Fitzsimmons, C. (2019). *Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle*. 1–17.

Li, G., Li, J., Kohl, K. D., Yin, B., Wei, W., Wan, X., Zhu, B., & Zhang, Z. (2019). Dietary shifts influenced by livestock grazing shape the gut microbiota composition and co-occurrence networks in a local rodent species. *Journal of Animal Ecology*, *88*(2), 302–314. https://doi.org/10.1111/1365-2656.12920

Li, L., Renaud, D. L., Goetz, H. M., Jessop, E., Costa, M. C., Gamsjäger, L., & Gomez, D. E. (2023). Effect of time of sample collection after onset of diarrhea on fecal microbiota composition of calves. *Journal of Veterinary Internal Medicine*, *37*(4), 1588–1593. https://doi.org/10.1111/jvim.16801

Li, M., Wang, Z., Wang, L., Xue, B., Hu, R., & Zou, H. (2022). *Comparison of changes in fecal microbiota of calves with and without dam*. https://doi.org/10.7717/peerj.12826

Li, R. W., Connor, E. E., Li, C., Baldwin Vi, R. L., & Sparks, M. E. (2012). Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environmental Microbiology*, *14*(1), 129–139. https://doi.org/10.1111/j.1462-2920.2011.02543.x

Lima, S. F., Teixeira, A. G. V, Lima, F. S., Ganda, E. K., Higgins, C. H., Oikonomou, G., & Bicalho, R. C. (2017). The bovine colostrum microbiome and its association with clinical mastitis. *Journal of Dairy Science*, *100*(4), 3031–3042. https://doi.org/10.3168/jds.2016-11604

Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Mackie, R. I., Sghir, A., & Gaskins, H. R. (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. *American Journal of Clinical Nutrition*, *69*(5), 1035–1045.

Malmuthuge, N. (2016). *Role of Commensal Microbiota in Neonatal Calf Gut Development*. https://doi.org/https://doi.org/10.3929/ethz-b-000238666

Malmuthuge, N., Chen, Y., Liang, G., Goonewardene, L. A., & Guan, L. L. (2015). Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. *Journal of Dairy Science*, *98*(11), 8044–8053. https://doi.org/10.3168/jds.2015-9607

Malmuthuge, N., & Griebel, P. J. (2019). A Novel Animal Model for Regional Microbial Dysbiosis of the Pioneer Microbial Community. *Frontiers in Microbiology*, *10*(July). https://doi.org/10.3389/fmicb.2019.01706

Malmuthuge, N., Griebel, P. J., & Guan, L. L. (2015). The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. *Frontiers in Veterinary Science*, *2*(36), 1–10. https://doi.org/10.3389/fvets.2015.00036

Malmuthuge, N., & Guan, L. L. (2017). Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health. *Journal of Dairy Science*, *100*(7), 5996–6005. https://doi.org/10.3168/jds.2016-12239

Malmuthuge, N., Li, M., Goonewardene, L. A., Oba, M., & Guan, L. L. (2013). Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. *Journal of Dairy Science*, *96*(5), 3189–3200. https://doi.org/10.3168/jds.2012-6200

Malmuthuge, N., Liang, G., Griebel, P. J., & Guan, L. L. (2019). Taxonomic and functional compositions of the small intestinal microbiome in neonatal calves provide a framework for understanding early life gut health. *Applied and Environmental Microbiology*, *85*(6), e02534-18. https://doi.org/10.1128/AEM.02534-18

Mao, S., Zhang, M., Liu, J., & Zhu, W. (2015). Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: Membership and potential function. *Scientific Reports*,

5(April), 1–14. https://doi.org/10.1038/srep16116

Mardis, E. R. (2013). Next-Generation Sequencing Platforms. *Annual Review of Analytical Chemistry*, *6*(1), 287–303. https://doi.org/10.1146/annurev-anchem-062012-092628

Maynard, C. L., Elson, C. O., Hatton, R. D., & Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature*, *489*(7415), 231–241. https://doi.org/10.1038/nature11551

Meale, S. J., Chaucheyras-Durand, F., Berends, H., Guan, L. L., & Steele, M. A. (2017). From pre- to postweaning: Transformation of the young calf's gastrointestinal tract. *Journal of Dairy Science*, *100*(7), 5984–5995. https://doi.org/10.3168/jds.2016-12474

Meale, S. J., Li, S., Azevedo, P., Derakhshani, H., Plaizier, J. C., Khafipour, E., & Steele, M. A. (2016). Development of ruminal and fecal microbiomes are affected by weaning but not weaning strategy in dairy calves. *Frontiers in Microbiology*, *7*(582), 1–16. https://doi.org/10.3389/fmicb.2016.00582

Messer, J. S., & Chang, E. B. (2018). Microbial Physiology of the Digestive Tract and Its Role in Inflammatory Bowel Diseases. In *Physiology of the Gastrointestinal Tract* (Sixth Edit). Elsevier Inc. https://doi.org/10.1016/B978-0-12-809954-4.00036-0

Millemann, Y. (2009). Diagnosis of neonatal calf diarrhoea. *Revue de Medecine Veterinaire*, *160*(8–9), 404–409.

Mizrahi-Man, O., Davenport, E. R., & Gilad, Y. (2013). Taxonomic Classification of Bacterial 16S rRNA Genes Using Short Sequencing Reads: Evaluation of Effective Study Designs. *PLoS ONE*, *8*(1), 18–23. https://doi.org/10.1371/journal.pone.0053608

Mizrahi, I., & Jami, E. (2018). Review: The compositional variation of the rumen microbiome and its effect on host performance and methane emission. *Animal*, *12*(2), S220–S232. https://doi.org/10.1017/S1751731118001957

Moor, J., Wüthrich, T., Aebi, S., Mostacci, N., Overesch, G., Oppliger, A., & Hilty, M. (2021). Influence of pig farming on human Gut Microbiota: role of airborne microbial communities.

Gut Microbes, *13*(1), 1–13. https://doi.org/10.1080/19490976.2021.1927634

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., Kitzman, D. W., Kushugulova, A., Marotta, F., & Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging*, *4*(4), 267–285. https://doi.org/10.3233/NHA-170030

Obregon-Gutierrez, P., Bague-Companys, J., Bach, A., Aragon, V., & Correa-Fiz, F. (2022). Longitudinal Study of Fecal Microbiota in Calves with or without Diarrhea Episodes before Weaning. *Veterinary Sciences*, *9*(9). https://doi.org/10.3390/vetsci9090463

Oikonomou, G., Teixeira, A. G. V., Foditsch, C., Bicalho, M. L., Machado, V. S., & Bicalho, R. C. (2013). Fecal Microbial Diversity in Pre-Weaned Dairy Calves as Described by Pyrosequencing of Metagenomic 16S rDNA. Associations of Faecalibacterium Species with Health and Growth. *PLoS ONE*, *8*(4), e63157. https://doi.org/10.1371/journal.pone.0063157

Ozutsumi, Y., Hayashi, H., Sakamoto, M., Itabashi, H., & Benno, Y. (2005). Culture-independent analysis of fecal microbiota in cattle. *Bioscience, Biotechnology and Biochemistry*, *69*(9), 1793–1797. https://doi.org/10.1271/bbb.69.1793

Pelzer, E., Gomez-Arango, L. F., Barrett, H. L., & Nitert, M. D. (2017). Review: Maternal health and the placental microbiome. *Placenta*, *54*, 30–37. https://doi.org/10.1016/j.placenta.2016.12.003

Pflughoeft, K. J., Mash, M., Hasenkampf, N. R., Jacobs, M. B., Tardo, A. C., Magee, D. M., Song, L., Labaer, J., Philipp, M. T., Embers, M. E., & Aucoin, D. P. (2019). *Multi-platform Approach for Microbial Biomarker Identification Using Borrelia burgdorferi as a Model*. *9*(June), 1–10. https://doi.org/10.3389/fcimb.2019.00179

Proctor, L., Ravel, J., & Turnbaugh, P. (2013). FAQ: Human Microbiome. In *FAQ: Human Microbiome* (Vol. 1, Issue 1). http://www.ncbi.nlm.nih.gov/pubmed/33054142

Rajendhran, J., & Gunasekaran, P. (2011). Microbial phylogeny and diversity: Small subunit ribosomal RNA sequence analysis and beyond. In *Microbiological Research*. https://doi.org/10.1016/j.micres.2010.02.003

Rey, M., Enjalbert, F., Combes, S., Cauquil, L., Bouchez, O., & Monteils, V. (2014). Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. *Journal of Applied Microbiology*, *116*(2), 245–257. https://doi.org/10.1111/jam.12405

Russell, J. B., & Rychlik, J. L. (2001). Factors That Alter Rumen Microbial Ecology. *Science*, *292*(5519), 1119–1122. http://www.sciencemag.org/cgi/content/abstract/292/5519/1119

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8), 1–14. https://doi.org/10.1371/journal.pbio.1002533

Shams, M. H., Hashemzadeh, F., Khorvash, M., Pazoki, A., & Beiranvand, H. (2022). Interaction of colostrum pasteurization with probiotics supplementation on health and performance of Holstein calves. *Animal Feed Science and Technology*, *288*(May), 115319. https://doi.org/10.1016/j.anifeedsci.2022.115319

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology*, *11*(4), 227–238. https://doi.org/10.1038/nrmicro2974

Sudatip, D., Mostacci, N., Thamlikitkul, V., Oppliger, A., & Hilty, M. (2022). Influence of occupational exposure to pigs or chickens on human gut microbiota composition in Thailand. *One Health*, *15*(July), 100463. https://doi.org/10.1016/j.onehlt.2022.100463

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The Role of Short-Chain Fatty Acids in Health and Disease. In *Advances in Immunology* (1st ed., Vol. 121). Elsevier Inc. https://doi.org/10.1016/B978-0-12-800100-4.00003-9

Theelen, M. J. P., Luiken, R. E. C., Wagenaar, J. A., Sloet van Oldruitenborgh-Oosterbaan, M. M., Rossen, J. W. A., & Zomer, A. L. (2021). The equine faecal microbiota of healthy horses and ponies in the netherlands: Impact of host and environmental factors. *Animals*, *11*(6), 1–17. https://doi.org/10.3390/ani11061762

Timmerman, H. M., Mulder, L., Everts, H., Van Espen, D. C., Van Der Wal, E., Klaassen, G., Rouwers, S. M. G., Hartemink, R., Rombouts, F. M., & Beynen, A. C. (2005). Health and growth of veal calves fed milk replacers with or without probiotics. *Journal of Dairy Science*, *88*(6), 2154–2165. https://doi.org/10.3168/jds.S0022-0302(05)72891-5

Tizard, I. R. (1993). Veterinary Immunology: An Introduction. In *Saunders* (Vol. 70, Issue 10). https://doi.org/10.1111/j.1751-0813.1993.tb00833.x

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. a, Jason, P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R., Gordon, J. I., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2009). A core gut microbiome between lean and obesity twins. *Nature*, *457*(7228), 480–484. https://doi.org/10.1038/nature07540.A

Uddin, M. S., Schwartzkopf-Genswein, K. S., Waldner, M., Meléndez, D. M., Niu, Y. D., & Alexander, T. W. (2023). Auction market placement and a rest stop during transportation affect the respiratory bacterial microbiota of beef cattle. *Frontiers in Microbiology*, *14*(September), 1–16. https://doi.org/10.3389/fmicb.2023.1192763

Urie, N. J., Lombard, J. E., Shivley, C. B., Kopral, C. A., Adams, A. E., Earleywine, T. J., & Olson, J. D. (2020). *Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves*. *January*.

Uyeno, Y., Sekiguchi, Y., & Kamagata, Y. (2010). rRNA-based analysis to monitor succession of faecal bacterial communities in Holstein calves. *Letters in Applied Microbiology*, *51*(5), 570–577. https://doi.org/10.1111/j.1472-765X.2010.02937.x

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *British Medical Association (Online)*, *361*, 36–44. https://doi.org/10.1136/bmj.k2179

Varshney, J., & Naresh, R. (2005). *Comparative efficacy of homeopathic and allopathic systems of medicine in the management of clinical mastitis of Indian dairy cows*. 81–85. https://doi.org/10.1016/j.homp.2004.11.013

Weese, J. S., Holcombe, S. J., Embertson, R. M., Kurtz, K. A., Roessner, H. A., Jalali, M., & Wismer, S. E. (2015). Changes in the faecal microbiota of mares precede the development of post partum colic. *Equine Veterinary Journal*, *47*(6), 641–649. https://doi.org/10.1111/evj.12361

Weese, J. S., & Jelinski, M. (2017). Assessment of the Fecal Microbiota in Beef Calves. *Journal of Veterinary Internal Medicine*, *31*(1), 176–185. https://doi.org/10.1111/jvim.14611

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Yeoman, C. J., Ishaq, S. L., Bichi, E., Olivo, S. K., Lowe, J., & Aldridge, B. M. (2018). Biogeographical Differences in the Influence of Maternal Microbial Sources on the Early Successional Development of the Bovine Neonatal Gastrointestinal tract. *Scientific Reports*, *8*(3197), 1–14. https://doi.org/10.1038/s41598-018-21440-8

Zeineldin, M., Aldridge, B., & Lowe, J. (2018). Dysbiosis of the fecal microbiota in feedlot cattle with hemorrhagic diarrhea. *Microbial Pathogenesis*, *115*, 123–130. https://doi.org/10.1016/j.micpath.2017.12.059

Zhao, L., Li, X., Atwill, E. R., Aly, S. S., Williams, D. R., & Su, Z. (2022). Dynamic changes in fecal bacterial microbiota of dairy cattle across the production line. *BMC Microbiology*, *22*(1), 1–15. https://doi.org/10.1186/s12866-022-02549-3

Chapter 3 - General discussion

The GIT microbiota has been a topic of immense interest over the last years, as its composition and diversity seem to be intimately linked to health and disease. A higher fecal microbial diversity is associated to a more efficient system (Larsen & Claassen, 2018) and imbalances in the GIT microbiota such as diarrhea episodes are associated to lower diversity and disease. Interestingly, in our study an increased diversity and richness were observed on days when the diarrhea proportion were higher. We assume that this increase in diversity can be associated to the animal age, diet changes or adaptation to the new environment, when the GIT microbiota diversity can increases in terms of *alpha* diversity (Dill-Mcfarland et al., 2017b) and not to health status.

The *beta*-diversity changed during the study, but surprisingly the changes were according to days and not according to healthy status (presence or absence of diarrhea). We speculate that the administration of antimicrobials and or diarrhea episodes delayed the temporal development of diversity and taxa–function robustness and the changes observed were age associated as well.

Thus, fully understanding the diversity of the gut community in animals, and how these populations and communities relate to animal performance and pathogen infections in livestock is crucial to making improvements in animal health and productivity.

Diarrhea is the leading cause of morbidity, mortality, and antimicrobial resistance in calves during the first months of age, moreover diarrhea is associated to several changes in the bacterial communities composition in the GIT (Gomez et al., 2022). The decrease in the relative abundance of some genera during the study (i.e., *Bifidobacterium*, *Lactobacillus* and *Faecalibacterium*) are related to gastrointestinal dysbiosis once these a high abundance of these bacteria are well known to be associated to health in calves (Dong et al., 2021; Du et al., 2023; L. Li et al., 2023).

Manipulation of the gastrointestinal microbiota has been investigated to improve animal production efficiency. To date, these efforts have been hampered by the settled and resilient nature of the adult GIT microbiota which makes it refractory to persistent change. In the first weeks of life, health calves harbour a more heterogenous microbiota when compared to adult

ruminants (Jami et al., 2013). This timeframe seems to be a good opportunity to manipulate the microbial composition and function in their developing GIT. A study attempted to improve calf health by manipulating early GIT microbial composition with oral supplementation of Faecalibacterium prausnitzii (Foditsch et al., 2015), a bacterium reported to be negatively associated with calf diarrhea (Oikonomou et al., 2013). Oral administration of F. prausnitzii during the first week of life effectively reduced the incidence of diarrhea and calf death related to diarrhea in preweaned calves during the first 7 weeks of life (Foditsch et al., 2015). As well, F. prausnitzii was more abundant in treated calves during the first 5 weeks of life than in control calves (Foditsch et al., 2015), indicating that early microbial interventions persist within the GIT, and that this persistence may play a role in influencing host health.

Dairy calves have an immature GIT at birth, with a non-functional rumen. The rumen proportions are smaller than in adult cows and structures as rumen wall *villi* which are essentials for nutrient absorption are not developed yet. During the first three weeks of life, milk, the major component of diet is directly carried by the oesophageal groove into the abomasum without passing by the rumen (Meale et al., 2017).

The study presented provide valuable fundamental information concerning the GIT microbiota composition of calves arriving to a rearing unit from different farms and how transportation, dietary change, age, and environment can impact the richness and diversity of calf's microbiota especially in its first weeks of life.

Identifying the window for effective early-life manipulation first requires a full understanding of microbial colonisation and establishment dynamics in the developing GIT, and of the factors that influence it. Indeed, the establishment of the intestinal microbiota of calves is a complex process influenced by internal and external factors such as microbiota succession and understanding the dynamics of microbial establishment in the developing GIT and the factors that influence it will be key in the design an effective dietary management strategy to manipulate microbial composition, function and hence host performance in the future.

Limitations of this study include the lack of information about calves' origin (farm of origin, health status before arriving at the rearing unit, vaccination, type of transportation and duration,

distance of farm and auction, diet (milk, milk replacer, colostrum uptake) and antimicrobial administration). We assume that the animals used in this study did not receive antibiotics before arrival, as required and informed by the farm staff.

The identification of microorganisms (biomarkers) in calves' feces would be helpful, for example, to identify individual calves at an early stage with an enhance probability to develop dysbiosis/diarrhea, and to take preventive measures before clinical signs occur. Thus, studies to explore variations in the early microbial colonization may be valuable to better understand the role of the initial microbiota in subsequent colonization, succession, and GIT development. This knowledge will also help and reveal a means to manipulate the microbial colonization process by modifying early management practices for calves.

References

Alipour, M. J., Jalanka, J., Pessa-Morikawa, T., Kokkonen, T., Satokari, R., Hynönen, U., Iivanainen, A., & Niku, M. (2018). The composition of the perinatal intestinal microbiota in cattle. *Scientific Reports*, *8*(10437), 1–14. https://doi.org/10.1038/s41598-018-28733-y

AlShawaqfeh, M. K., Wajid, B., Minamoto, Y., Markel, M., Lidbury, J. A., Steiner, J. M., Serpedin, E., & Suchodolski, J. S. (2017). A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. FEMS Microbio, M. K., Wajid, B., Minamoto, Y., Markel, M., Lidbury, J. A., Steiner, J. M., Serpedin, E., & Suchodolski, J. S. (2017). A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiology Ecology*, *93*(11), 1–8. https://doi.org/10.1093/femsec/fix136

Amin, N., & Seifert, J. (2021). Dynamic progression of the calf ' s microbiome and its influence on host health. *Computational and Structural Biotechnology Journal*, *19*, 989–1001. https://doi.org/10.1016/j.csbj.2021.01.035

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., Versalovic, J., Young, V., & Finlay, B. B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host and Microbe*, *12*(5), 611–622. https://doi.org/10.1016/j.chom.2012.10.012

Bager, P., Wohlfahrt, J., & Westergaard, T. (2008). Caesarean delivery and risk of atopy and allergic disesase: Meta-analyses. *Clinical and Experimental Allergy*, *38*(4), 634–642. https://doi.org/10.1111/j.1365-2222.2008.02939.x

Barko, P. C., McMichael, M. A., Swanson, K. S., & Williams, D. A. (2018). The Gastrointestinal Microbiome: A Review. *Journal of Veterinary Internal Medicine*, *32*(1), 9–25. https://doi.org/10.1111/jvim.14875

Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Bignell, H. R., & Boutell, J. M. (2008). An improved ant colony algorithm used for unscented Kalman filter. *Accurate Whole*

Human Genome Sequencing Using Reversible Terminator Chemistry, *6*(456 (7218)), 53–59. https://doi.org/10.1038/nature07517.Accurate

Cani, P. D., Van Hul, M., Lefort, C., Depommier, C., Rastelli, M., & Everard, A. (2019). Microbial regulation of organismal energy homeostasis. *Nature Metabolism*, *1*(1), 34–46. https://doi.org/10.1038/s42255-018-0017-4

Chen, H., Liu, Y., Huang, K., Yang, B., Zhang, Y., Yu, Z., & Wang, J. (2022). Fecal microbiota dynamics and its relationship to diarrhea and health in dairy calves. *Journal of Animal Science and Biotechnology*, *13*(1), 1–20. https://doi.org/10.1186/s40104-022-00758-4

Choudhury, P. H., Salem, A. Z. M., Jena, R., Kumar, S., Singh, R., & Puniya, A. kumar. (2015). Rumen microbiology: From evolution to revolution. In *Rumen Microbiology: From Evolution to Revolution*. https://doi.org/10.1007/978-81-322-2401-3

Constable, P. D. (2004). Antimicrobial Use in the Treatment of Calf Diarrhea Change in Small Intestinal Bacterial Flora in Calves with Diarrhea. *J Vet Intern Med*, *18*, 8–17.

Costa, M. C., & Weese, J. S. (2019). Methods and basic concepts for microbiota assessment. *Veterinary Journal*, *249*, 10–15. https://doi.org/10.1016/j.tvjl.2019.05.005

Costa, M., Stämpfli, H. R., Allen-Vercoe, E., & Weese, J. S. (2015). Development of the faecal microbiota in foals. *Equine Veterinary Journal*, *0*, 1–8. https://doi.org/10.1111/evj.12532

Costa, M., & Weese, J. S. (2019). Methods and basic concepts for microbiota assessment. *Veterinary Journal*, *249*, 10–15. https://doi.org/10.1016/j.tvjl.2019.05.005

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J. M., & Relman, D. A. (2012). The application of ecological theory towrds an understanding of the human microbiome. *Science*, *336*(6086), 1255–1262. https://doi.org/10.1126/science.1224203.The

de Tejada, B. M. (2014). Antibiotic use and misuse during pregnancy and delivery: Benefits and risks. *International Journal of Environmental Research and Public Health*, *11*(8), 7993–8009. https://doi.org/10.3390/ijerph110807993

Dempsey, E., & Corr, S. C. (2022). Lactobacillus spp. for Gastrointestinal Health: Current and

Future Perspectives. *Frontiers in Immunology*, *13*(March 2020), 1–15. https://doi.org/10.3389/fimmu.2022.840245

Diao, Q., Zhang, R., & Fu, T. (2019). *Review of Strategies to Promote Rumen Development in Calves*. 1–15.

Dias, J., Marcondes, I., Souza, M. De, Noronha, M. F., Resende, T., Machado, S., & Mantovani, C. (2018). Bacterial Community Dynamics across the Gastrointestinal Tracts of Dairy Calves during Preweaning Development. *Applied and Environmental Microbiology*, *84*(9), 1–12.

Dill-Mcfarland, K. A., Breaker, J. D., & Suen, G. (2017a). Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation. *Scientific Reports*, *7*(June 2016), 1–12. https://doi.org/10.1038/srep40864

Dill-Mcfarland, K. A., Breaker, J. D., & Suen, G. (2017b). Microbial succession in the gastrointestinal tract of dairy cows from 2 weeks to first lactation. *Scientific Reports*, *7*(January), 1–12. https://doi.org/10.1038/srep40864

Dill-McFarland, K. A., Weimer, P. J., Breaker, J. D., & Suen, G. (2019). Diet Influences Early Microbiota Development in Dairy Calves without Long-Term Impacts on Milk Production. *Applied and Environmental Microbiology*, *85*(2), 1–12. https://doi.org/10.1128/AEM.02141-18

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107

Dong, H., Liu, B., Li, A., Iqbal, M., Mehmood, K., Jamil, T., Chang, Y. F., Zhang, H., & Wu, Q. (2021). Microbiome Analysis Reveals the Attenuation Effect of Lactobacillus From Yaks on Diarrhea via Modulation of Gut Microbiota. *Frontiers in Cellular and Infection Microbiology*, *10*(February), 1–13. https://doi.org/10.3389/fcimb.2020.610781

Du, W., Wang, X., Hu, M., Hou, J., Du, Y., Si, W., Yang, L., Xu, L., & Xu, Q. (2023). Modulating

gastrointestinal microbiota to alleviate diarrhea in calves. *Frontiers in Microbiology*, *14*(June), 1–13. https://doi.org/10.3389/fmicb.2023.1181545

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., & Relman, D. A. (2005). Diversity of the Human Intestinal Microbial Flora. *Science*, *308*(5728), 1635–1638. https://doi.org/10.1126/science.1110591

Eisler, M. C., Lee, M. R. F., Tarlton, J. F., & Martin, G. B. (2014). Steps to sustainable livestock. *Nature*, *507*(7490), 32–34. https://doi.org/10.1038/507032a

Fan, P., Bian, B., Teng, L., Nelson, C. D., Driver, J., Elzo, M. A., & Jeong, K. C. (2020). Host genetic effects upon the early gut microbiota in a bovine model with graduated spectrum of genetic variation. *ISME Journal*, *14*(1), 302–317. https://doi.org/10.1038/s41396-019-0529-2

Garcia Dias, A. L., & Ametaj, B. N. (2017). Cattle Gastrointestinal Tract Microbiota in Health and Disease. In *Periparturient Diseases of Dairy Cows: A Systems Biology Approach* (Vol. 1, pp. 71–91). https://doi.org/10.1007/978-3-319-43033-1

Gensollen, T., Iyer, S. S., Kasper, D. L., Blumberg, R. S., & Medical, H. (2016). How colonization by microbiota in early life shapes the immune 1. Gensollen T, Iyer SS, Kasper DL, Blumberg RS, Medical H. How colonization by microbiota in early life shapes the immune system. Science. 2016;352(6285):539-544. doi:10.1126/science.aad9378. *Science (New York, N.Y.)*, *352*(6285), 539–544. https://doi.org/10.1126/science.aad9378.How

Gibbons, J. F., Boland, F., Buckley, J. F., Butler, F., Egan, J., Fanning, S., Markey, B. K., & Leonard, F. C. (2013). *Influences on antimicrobial prescribing behaviour of veterinary practitioners in cattle practice in Ireland*. https://doi.org/10.1136/vr.100782

Gomez, D. E., Arroyo, L. G., Costa, M. C., Viel, L., & Weese, J. S. (2017). Characterization of the Fecal Bacterial Microbiota of Healthy and Diarrheic Dairy Calves. *Journal of Veterinary Internal Medicine*, *31*(3), 928–939. https://doi.org/10.1111/jvim.14695

Gomez, D. E., Galvão, K. N., Rodriguez-Lecompte, J. C., & Costa, M. C. (2019). The Cattle Microbiota and the Immune System: An Evolving Field. *Veterinary Clinics of North America - Food Animal Practice*, *35*(3), 485–505. https://doi.org/10.1016/j.cvfa.2019.08.002

Gomez, D. E., Li, L., Goetz, H., Macnicol, J., Gamsjaeger, L., & Renaud, D. L. (2022). *Calf Diarrhea Is Associated With a Shift From Obligated to Facultative Anaerobes and Expansion of Lactate-Producing Bacteria*. *9*(March), 1–8. https://doi.org/10.3389/fvets.2022.846383

Govil, K., Yadav, D. S., Patil, A. K., Nayak, S., Baghel, R. P. S., & Yadav, P. K. (2017). *Feeding management for early rumen development in calves*. *5*(3), 1132–1139.

Hang, B. P. T., Wredle, E., & Dicksved, J. (2021). Analysis of the developing gut microbiota in young dairy calves—impact of colostrum microbiota and gut disturbances. *Tropical Animal Health and Production*, *53*(1). https://doi.org/10.1007/s11250-020-02535-9

Hartinger, T., Pacífico, C., Poier, G., Terler, G., & Klevenhusen, F. (2022). Shift of dietary carbohydrate source from milk to various solid feeds reshapes the rumen and fecal microbiome in calves. *Scientific Reports*, 1–12. https://doi.org/10.1038/s41598-022-16052-2

Hasan, N., & Yang, H. (2019). Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ*, *2019*(8), 1–31. https://doi.org/10.7717/peerj.7502

He, Y., Maltecca, C., & Tiezzi, F. (2021). Potential use of gut microbiota composition as a biomarker of heat stress in monogastric species: A review. *Animals*, *11*(6). https://doi.org/10.3390/ani11061833

Heinrichs, J. (2005). *Rumen Development in the Dairy Calf Rudimentary Reticulo-Rumen Changes in Rumen Epithelium*. *17*, 179–187.

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., & Janssen, P. H. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*, *5*(14567), 1–13. https://doi.org/10.1038/srep14567

Holman, D. B., Yang, W., & Alexander, T. W. (2019). Antibiotic treatment in feedlot cattle: A longitudinal study of the effect of oxytetracycline and tulathromycin on the fecal and nasopharyngeal microbiota. *Microbiome*, *7*(1), 1–14. https://doi.org/10.1186/s40168-019-0696-4

Holmes, A. H., Moore, L. S. P., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. V. (2015). *Antimicrobials : access and sustainable eff ectiveness 2 Understanding the mechanisms and drivers of antimicrobial resistance*. *6736*(15). https://doi.org/10.1016/S0140-6736(15)00473-0

Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjölund-Karlsson, M., Jansson, J. K., & Engstrand, L. (2010). Short-term antibiotic treatment has differing long- term impacts on the human throat and gut microbiome. *PLoS ONE*, *5*(3). https://doi.org/10.1371/journal.pone.0009836

Jami, E., Israel, A., Kotser, A., & Mizrahi, I. (2013). Exploring the bovine rumen bacterial community from birth to adulthood. *International Society for Microbial Ecology*, *7*(6), 1069–1079. https://doi.org/10.1038/ismej.2013.2

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., & Sasikala, M. (2015). *role of the normal gut microbiota*. *21*(29), 8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787

Kamada, N., Chen, G. Y., Inohara, N., & Núñez, G. (2013). *Control of pathogens and pathobionts by the gut microbiota*. *14*(7), 685–690. https://doi.org/10.1038/ni.2608

Kamra, D. N. (2005). Rumen Microbial Ecosystem. *Current Science*, *89*(1), 124–135. https://doi.org/10.1146/annurev.es.06.110175.000351

Kelly, D., King, T., & Aminov, R. (2007). Importance of microbial colonization of the gut in early life to the development of immunity. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, *622*(1–2), 58–69. https://doi.org/10.1016/j.mrfmmm.2007.03.011

Khalil, A., Batool, A., & Arif, S. (2022). Healthy Cattle Microbiome and Dysbiosis in Diseased Phenotypes. *Ruminants*, *2*(1), 134–156. https://doi.org/10.3390/ruminants2010009

Kim, M., Kim, J., Kuehn, L. A., Bono, J. L., Berry, E. D., Kalchayanand, N., Freetly, H. C., Benson, A. K., & Wells, J. E. (2014). Investigation of bacterial diversity in the feces of cattle fed different diets. *Journal of Animal Science*, *92*(2), 683–694. https://doi.org/10.2527/jas2013-6841

Klein-Jöbstl, D., Iwersen, M., & Drillich, M. (2014). Farm characteristics and calf management

practices on dairy farms with and without diarrhea: A case-control study to investigate risk factors for calf diarrhea. *Journal of Dairy Science*, *97*(8), 5110–5119. https://doi.org/10.3168/jds.2013-7695

Klein-Jöbstl, D., Quijada, N. M., Dzieciol, M., Feldbacher, B., Wagner, M., Drillich, M., Schmitz-Esser, S., & Mann, E. (2019). Microbiota of newborn calves and their mothers reveals possible transfer routes for newborn calves' gastrointestinal microbiota. *Plos One*, *14*(8), e0220554. https://doi.org/10.1371/journal.pone.0220554

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), 1–11. https://doi.org/10.1093/nar/gks808

Kolbert, C. P., & Persing, D. H. (1999). Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Current Opinion in Microbiology*, *2*(3), 299–305. https://doi.org/10.1016/S1369-5274(99)80052-6

Kostic, A. D., Ramink, X. J., & Dirk, G. (2014). The Microbiome in Inflammatory Bowel Diseases: Current Status and the Future Ahead. *Gastroenterology*, *146*(6), 1489–1499. https://doi.org/10.1053/j.gastro.2014.02.009.The

Kraimi, N., Dawkins, M., Gebhardt-Henrich, S. G., Velge, P., Rychlik, I., Volf, J., Creach, P., Smith, A., Colles, F., & Leterrier, C. (2019). Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: A review. *Physiology & Behavior*, *210*(112658), 1–12. https://doi.org/10.1016/j.physbeh.2019.112658

Kuo, C. H., Kuo, H. F., Huang, C. H., Yang, S. N., Lee, M. S., & Hung, C. H. (2013). Early life exposure to antibiotics and the risk of childhood allergic diseases: An update from the perspective of the hygiene hypothesis. *Journal of Microbiology, Immunology and Infection*, *46*(5), 320–329. https://doi.org/10.1016/j.jmii.2013.04.005

Larsen, O. F. A., & Claassen, E. (2018). The mechanistic link between health and gut microbiota diversity. *Scientific Reports*, *8*(1), 6–10. https://doi.org/10.1038/s41598-018-20141-6

Leeming, E. R., Johnson, A. J., Spector, T. D., & Roy, C. I. Le. (2019). Effect of Diet on the Gut Microbiota: Rethinking Intervention Duration. *Nutrients*, *11*(2862), 1–28.

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. *Nature Reviews Immunology*, *17*(4), 219–232. https://doi.org/10.1038/nri.2017.7

Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., & Fitzsimmons, C. (2019). *Host genetics influence the rumen microbiota and heritable rumen microbial features associate with feed efficiency in cattle*. 1–17.

Li, G., Li, J., Kohl, K. D., Yin, B., Wei, W., Wan, X., Zhu, B., & Zhang, Z. (2019). Dietary shifts influenced by livestock grazing shape the gut microbiota composition and co-occurrence networks in a local rodent species. *Journal of Animal Ecology*, *88*(2), 302–314. https://doi.org/10.1111/1365-2656.12920

Li, L., Renaud, D. L., Goetz, H. M., Jessop, E., Costa, M. C., Gamsjäger, L., & Gomez, D. E. (2023). Effect of time of sample collection after onset of diarrhea on fecal microbiota composition of calves. *Journal of Veterinary Internal Medicine*, *37*(4), 1588–1593. https://doi.org/10.1111/jvim.16801

Li, M., Wang, Z., Wang, L., Xue, B., Hu, R., & Zou, H. (2022). *Comparison of changes in fecal microbiota of calves with and without dam*. https://doi.org/10.7717/peerj.12826

Li, R. W., Connor, E. E., Li, C., Baldwin Vi, R. L., & Sparks, M. E. (2012). Characterization of the rumen microbiota of pre-ruminant calves using metagenomic tools. *Environmental Microbiology*, *14*(1), 129–139. https://doi.org/10.1111/j.1462-2920.2011.02543.x

Lima, S. F., Teixeira, A. G. V, Lima, F. S., Ganda, E. K., Higgins, C. H., Oikonomou, G., & Bicalho, R. C. (2017). The bovine colostrum microbiome and its association with clinical mastitis. *Journal of Dairy Science*, *100*(4), 3031–3042. https://doi.org/10.3168/jds.2016-11604

Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Mackie, R. I., Sghir, A., & Gaskins, H. R. (1999). Developmental microbial ecology of the neonatal gastrointestinal tract. *American Journal of Clinical Nutrition*, *69*(5), 1035–1045.

Malmuthuge, N. (2016). *Role of Commensal Microbiota in Neonatal Calf Gut Development*. https://doi.org/https://doi.org/10.3929/ethz-b-000238666

Malmuthuge, N., Chen, Y., Liang, G., Goonewardene, L. A., & Guan, L. L. (2015). Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. *Journal of Dairy Science*, *98*(11), 8044–8053. https://doi.org/10.3168/jds.2015-9607

Malmuthuge, N., & Griebel, P. J. (2019). A Novel Animal Model for Regional Microbial Dysbiosis of the Pioneer Microbial Community. *Frontiers in Microbiology*, *10*(July). https://doi.org/10.3389/fmicb.2019.01706

Malmuthuge, N., Griebel, P. J., & Guan, L. L. (2015). The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. *Frontiers in Veterinary Science*, *2*(36), 1–10. https://doi.org/10.3389/fvets.2015.00036

Malmuthuge, N., & Guan, L. L. (2017). Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health. *Journal of Dairy Science*, *100*(7), 5996–6005. https://doi.org/10.3168/jds.2016-12239

Malmuthuge, N., Li, M., Goonewardene, L. A., Oba, M., & Guan, L. L. (2013). Effect of calf starter feeding on gut microbial diversity and expression of genes involved in host immune responses and tight junctions in dairy calves during weaning transition. *Journal of Dairy Science*, *96*(5), 3189–3200. https://doi.org/10.3168/jds.2012-6200

Malmuthuge, N., Liang, G., Griebel, P. J., & Guan, L. L. (2019). Taxonomic and functional compositions of the small intestinal microbiome in neonatal calves provide a framework for understanding early life gut health. *Applied and Environmental Microbiology*, *85*(6), e02534-18. https://doi.org/10.1128/AEM.02534-18

Mao, S., Zhang, M., Liu, J., & Zhu, W. (2015). Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: Membership and potential function. *Scientific Reports*,

5(April), 1–14. https://doi.org/10.1038/srep16116

Mardis, E. R. (2013). Next-Generation Sequencing Platforms. *Annual Review of Analytical Chemistry*, *6*(1), 287–303. https://doi.org/10.1146/annurev-anchem-062012-092628

Maynard, C. L., Elson, C. O., Hatton, R. D., & Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature*, *489*(7415), 231–241. https://doi.org/10.1038/nature11551

Meale, S. J., Chaucheyras-Durand, F., Berends, H., Guan, L. L., & Steele, M. A. (2017). From pre- to postweaning: Transformation of the young calf's gastrointestinal tract. *Journal of Dairy Science*, *100*(7), 5984–5995. https://doi.org/10.3168/jds.2016-12474

Meale, S. J., Li, S., Azevedo, P., Derakhshani, H., Plaizier, J. C., Khafipour, E., & Steele, M. A. (2016). Development of ruminal and fecal microbiomes are affected by weaning but not weaning strategy in dairy calves. *Frontiers in Microbiology*, *7*(582), 1–16. https://doi.org/10.3389/fmicb.2016.00582

Messer, J. S., & Chang, E. B. (2018). Microbial Physiology of the Digestive Tract and Its Role in Inflammatory Bowel Diseases. In *Physiology of the Gastrointestinal Tract* (Sixth Edit). Elsevier Inc. https://doi.org/10.1016/B978-0-12-809954-4.00036-0

Millemann, Y. (2009). Diagnosis of neonatal calf diarrhoea. *Revue de Medecine Veterinaire*, *160*(8–9), 404–409.

Mizrahi-Man, O., Davenport, E. R., & Gilad, Y. (2013). Taxonomic Classification of Bacterial 16S rRNA Genes Using Short Sequencing Reads: Evaluation of Effective Study Designs. *PLoS ONE*, *8*(1), 18–23. https://doi.org/10.1371/journal.pone.0053608

Mizrahi, I., & Jami, E. (2018). Review: The compositional variation of the rumen microbiome and its effect on host performance and methane emission. *Animal*, *12*(2), S220–S232. https://doi.org/10.1017/S1751731118001957

Moor, J., Wüthrich, T., Aebi, S., Mostacci, N., Overesch, G., Oppliger, A., & Hilty, M. (2021). Influence of pig farming on human Gut Microbiota: role of airborne microbial communities.

Gut Microbes, *13*(1), 1–13. https://doi.org/10.1080/19490976.2021.1927634

Nagpal, R., Mainali, R., Ahmadi, S., Wang, S., Singh, R., Kavanagh, K., Kitzman, D. W., Kushugulova, A., Marotta, F., & Yadav, H. (2018). Gut microbiome and aging: Physiological and mechanistic insights. *Nutrition and Healthy Aging*, *4*(4), 267–285. https://doi.org/10.3233/NHA-170030

Obregon-Gutierrez, P., Bague-Companys, J., Bach, A., Aragon, V., & Correa-Fiz, F. (2022). Longitudinal Study of Fecal Microbiota in Calves with or without Diarrhea Episodes before Weaning. *Veterinary Sciences*, *9*(9). https://doi.org/10.3390/vetsci9090463

Oikonomou, G., Teixeira, A. G. V., Foditsch, C., Bicalho, M. L., Machado, V. S., & Bicalho, R. C. (2013). Fecal Microbial Diversity in Pre-Weaned Dairy Calves as Described by Pyrosequencing of Metagenomic 16S rDNA. Associations of Faecalibacterium Species with Health and Growth. *PLoS ONE*, *8*(4), e63157. https://doi.org/10.1371/journal.pone.0063157

Ozutsumi, Y., Hayashi, H., Sakamoto, M., Itabashi, H., & Benno, Y. (2005). Culture-independent analysis of fecal microbiota in cattle. *Bioscience, Biotechnology and Biochemistry*, *69*(9), 1793–1797. https://doi.org/10.1271/bbb.69.1793

Pelzer, E., Gomez-Arango, L. F., Barrett, H. L., & Nitert, M. D. (2017). Review: Maternal health and the placental microbiome. *Placenta*, *54*, 30–37. https://doi.org/10.1016/j.placenta.2016.12.003

Pflughoeft, K. J., Mash, M., Hasenkampf, N. R., Jacobs, M. B., Tardo, A. C., Magee, D. M., Song, L., Labaer, J., Philipp, M. T., Embers, M. E., & Aucoin, D. P. (2019). *Multi-platform Approach for Microbial Biomarker Identification Using Borrelia burgdorferi as a Model*. *9*(June), 1–10. https://doi.org/10.3389/fcimb.2019.00179

Proctor, L., Ravel, J., & Turnbaugh, P. (2013). FAQ: Human Microbiome. In *FAQ: Human Microbiome* (Vol. 1, Issue 1). http://www.ncbi.nlm.nih.gov/pubmed/33054142

Rajendhran, J., & Gunasekaran, P. (2011). Microbial phylogeny and diversity: Small subunit ribosomal RNA sequence analysis and beyond. In *Microbiological Research*. https://doi.org/10.1016/j.micres.2010.02.003

Rey, M., Enjalbert, F., Combes, S., Cauquil, L., Bouchez, O., & Monteils, V. (2014). Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. *Journal of Applied Microbiology*, *116*(2), 245–257. https://doi.org/10.1111/jam.12405

Russell, J. B., & Rychlik, J. L. (2001). Factors That Alter Rumen Microbial Ecology. *Science*, *292*(5519), 1119–1122. http://www.sciencemag.org/cgi/content/abstract/292/5519/1119

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8), 1–14. https://doi.org/10.1371/journal.pbio.1002533

Shams, M. H., Hashemzadeh, F., Khorvash, M., Pazoki, A., & Beiranvand, H. (2022). Interaction of colostrum pasteurization with probiotics supplementation on health and performance of Holstein calves. *Animal Feed Science and Technology*, *288*(May), 115319. https://doi.org/10.1016/j.anifeedsci.2022.115319

Sommer, F., & Bäckhed, F. (2013). The gut microbiota-masters of host development and physiology. *Nature Reviews Microbiology*, *11*(4), 227–238. https://doi.org/10.1038/nrmicro2974

Sudatip, D., Mostacci, N., Thamlikitkul, V., Oppliger, A., & Hilty, M. (2022). Influence of occupational exposure to pigs or chickens on human gut microbiota composition in Thailand. *One Health*, *15*(July), 100463. https://doi.org/10.1016/j.onehlt.2022.100463

Tan, J., McKenzie, C., Potamitis, M., Thorburn, A. N., Mackay, C. R., & Macia, L. (2014). The Role of Short-Chain Fatty Acids in Health and Disease. In *Advances in Immunology* (1st ed., Vol. 121). Elsevier Inc. https://doi.org/10.1016/B978-0-12-800100-4.00003-9

Theelen, M. J. P., Luiken, R. E. C., Wagenaar, J. A., Sloet van Oldruitenborgh-Oosterbaan, M. M., Rossen, J. W. A., & Zomer, A. L. (2021). The equine faecal microbiota of healthy horses and ponies in the netherlands: Impact of host and environmental factors. *Animals*, *11*(6), 1–17. https://doi.org/10.3390/ani11061762

Timmerman, H. M., Mulder, L., Everts, H., Van Espen, D. C., Van Der Wal, E., Klaassen, G., Rouwers, S. M. G., Hartemink, R., Rombouts, F. M., & Beynen, A. C. (2005). Health and growth of veal calves fed milk replacers with or without probiotics. *Journal of Dairy Science*, *88*(6), 2154–2165. https://doi.org/10.3168/jds.S0022-0302(05)72891-5

Tizard, I. R. (1993). Veterinary Immunology: An Introduction. In *Saunders* (Vol. 70, Issue 10). https://doi.org/10.1111/j.1751-0813.1993.tb00833.x

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Ley, R. E., Sogin, M. L., Jones, W. J., Roe, B. a, Jason, P., Egholm, M., Henrissat, B., Heath, A. C., Knight, R., Gordon, J. I., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2009). A core gut microbiome between lean and obesity twins. *Nature*, *457*(7228), 480–484. https://doi.org/10.1038/nature07540.A

Uddin, M. S., Schwartzkopf-Genswein, K. S., Waldner, M., Meléndez, D. M., Niu, Y. D., & Alexander, T. W. (2023). Auction market placement and a rest stop during transportation affect the respiratory bacterial microbiota of beef cattle. *Frontiers in Microbiology*, *14*(September), 1–16. https://doi.org/10.3389/fmicb.2023.1192763

Urie, N. J., Lombard, J. E., Shivley, C. B., Kopral, C. A., Adams, A. E., Earleywine, T. J., & Olson, J. D. (2020). *Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves*. *January*.

Uyeno, Y., Sekiguchi, Y., & Kamagata, Y. (2010). rRNA-based analysis to monitor succession of faecal bacterial communities in Holstein calves. *Letters in Applied Microbiology*, *51*(5), 570–577. https://doi.org/10.1111/j.1472-765X.2010.02937.x

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *British Medical Association (Online)*, *361*, 36–44. https://doi.org/10.1136/bmj.k2179

Varshney, J., & Naresh, R. (2005). *Comparative efficacy of homeopathic and allopathic systems of medicine in the management of clinical mastitis of Indian dairy cows*. 81–85. https://doi.org/10.1016/j.homp.2004.11.013

Weese, J. S., Holcombe, S. J., Embertson, R. M., Kurtz, K. A., Roessner, H. A., Jalali, M., & Wismer, S. E. (2015). Changes in the faecal microbiota of mares precede the development of post partum colic. *Equine Veterinary Journal*, *47*(6), 641–649. https://doi.org/10.1111/evj.12361

Weese, J. S., & Jelinski, M. (2017). Assessment of the Fecal Microbiota in Beef Calves. *Journal of Veterinary Internal Medicine*, *31*(1), 176–185. https://doi.org/10.1111/jvim.14611

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, *173*(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Yeoman, C. J., Ishaq, S. L., Bichi, E., Olivo, S. K., Lowe, J., & Aldridge, B. M. (2018). Biogeographical Differences in the Influence of Maternal Microbial Sources on the Early Successional Development of the Bovine Neonatal Gastrointestinal tract. *Scientific Reports*, *8*(3197), 1–14. https://doi.org/10.1038/s41598-018-21440-8

Zeineldin, M., Aldridge, B., & Lowe, J. (2018). Dysbiosis of the fecal microbiota in feedlot cattle with hemorrhagic diarrhea. *Microbial Pathogenesis*, *115*, 123–130. https://doi.org/10.1016/j.micpath.2017.12.059

Zhao, L., Li, X., Atwill, E. R., Aly, S. S., Williams, D. R., & Su, Z. (2022). Dynamic changes in fecal bacterial microbiota of dairy cattle across the production line. *BMC Microbiology*, *22*(1), 1–15. https://doi.org/10.1186/s12866-022-02549-3