Using the Microbiome to Enhance Health and Production in Food Producing Animals

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What is a microbiome and why is it important?

Mammals live in intimate contact with microorganisms that reside both in and on their body. The mammalian gut, for example, contains an estimated 100 trillion bacterial cells; this collection of microorganisms is generally referred to as the gut microbiota or microbiome. The term microbiota refers to the microorganisms that reside in a specific environment and microbiome is used when describing these microorganisms and their genetic content (Marchesi and Ravel, 2015). However, these terms are also often used interchangeably. Although bacteria are the predominant members in a typical host-associated microbiome, archaea, fungi, protozoa, and viruses are also present in most microbiomes. In humans and animals, the gut microbiome in particular plays an important role in health, including development and maintenance of the immune system (Round and Mazmanian, 2009). It also provides resistance against colonization from potentially pathogenic microorganisms (Lawley and Walker, 2013) and produces vitamins and shortchain fatty acids (e.g. acetate, butyrate, and propionate) from otherwise non-digestible carbohydrates like cellulose, xylan, and resistant starch (Nicholson et al., 2012). Consequently, anything that disrupts the gut microbiome can potentially negatively affect the health of the animal. As a result of this association with health and the widespread availability of high-throughput sequencing technology, microbiome research has flourished in recent years.

How are microbiomes characterized?

The fraction of bacteria that can be cultured from a specific microbiome varies but no single method can culture the large majority of bacteria. This has led to the development of high-throughput culture-independent technologies. In a typical microbiota experiment, total microbial DNA is extracted from a sample (e.g. feces, nasal swab) and a short segment of the bacterial 16S rRNA gene is targeted and amplified via polymerase chain reaction (PCR). The 16S rRNA gene is the most frequently chosen target because it is found in all bacteria and has nine hypervariable regions (V1 to V9), each of which is flanked by highly conserved regions. Universal 16S rRNA gene PCR primers targeting the conserved regions of the gene are then used to produce an amplicon library consisting of a short fragment that spans at least one 16S rRNA gene hypervariable region. These amplicon libraries are then sequenced and these sequences used to discern phylogenetic and taxonomic relationships among bacteria in the sample. Shotgun metagenomic sequencing is used to determine the total microbial gene content of the microbiome. In this method, rather than targeting a single gene, the entire extracted DNA is sequenced and analyzed. Although this technology can yield a great deal of information about a microbiome in terms of the functional potential, it is considerably more expensive and bioinformatically challenging to analyze than 16S rRNA gene sequencing. For this reason, most published studies to date have used 16S rRNA gene sequencing to analyze the microbiota/microbiome.

Microbiomes of food-producing animals

In recent years the microbiome has become a very active area of research in livestock sectors. As costs have continued to decline for high-throughput sequencing, more researchers have become involved in microbiota projects. As such, the gut microbiota of cattle, poultry, and swine have been well characterized to this point. In beef and dairy cattle, it is the rumen that is often the most microbial rich and diverse region in the gut (Mao et al., 2015) while microbial diversity is usually highest in the cecum and colon of monogastric animals such as swine (Holman et al., 2017) and in the cecum of chickens (Holman et al., 2017). Despite the variability among genera and species present in the gut microbiota of livestock, the phyla Bacteroidetes, Firmicutes, and Proteobacteria tend to be predominant among all food producing animals. As with humans, in many food-producing animals including cattle, pigs, and sheep, microbial colonization begins shortly after birth with microbes initially derived from the mother (Leser and Mølbak, 2009). In oviparous animals such as chickens, the gastrointestinal tract is colonized with microbes from the immediate environment

following hatching. The most significant factor affecting the composition of the gut microbiome in livestock is usually diet. In pigs for example, the change in diet that occurs following weaning results in large alterations in the gut microbial community (Holman and Chenier, 2014). In addition, the administration of antibiotics, environmental and physiological stress, and health status, can all affect the microbiomes of food producing animals.

Microbiome research to improve health and production in food producing animals

Traditionally, antimicrobials have been added to the feed of many food-producing animals to enhance growth, prevent disease, and improve feed efficiency. However, there are concerns about the relationship of antimicrobial use in livestock and the development of antimicrobial resistance. These concerns have brought about recent restrictions on antimicrobial use for the purpose of growth promotion in animals in the United States and similar restrictions are expected in Canada (Government of Canada, 2017). As a result, the need for antimicrobial alternatives has intensified. Although the underlying mechanism of antimicrobial growth promotion in livestock is unknown, it is generally thought to be due to a direct effect on the gut microbiome, either by inhibiting pathogens or by reducing the total microbial population in the gut (Allen et al., 2013). Some of the alternatives to antimicrobials that have been investigated in relation to the gut microbiome include prebiotics, probiotics, and organic acids.

Prebiotics are non-digestible substrates that preferentially affect the gut microbiome in a manner which benefits the host (Bindels et al., 2015). Inulin, fructooligosaccharides, and mannanoligosaccharides are among the prebiotic oligosaccharides commonly used. Probiotics, or direct-fed microbials, are live microorganisms, usually bacteria or yeast, that when administered impart a health benefit on the animal (Yeoman and White, 2014). Typical probiotics include Bacillus, Bifidobacterium, Enterococcus, Lactobacillus, and Streptococcus spp., as well as the yeast Saccharomyces cerevisiae (Jacela et al., 2009). Several products containing prebiotics and/or probiotics are already on the market for use in cattle, poultry, and swine production. Organic acids (e.g. butyric acid, citric acid, lactic acid) and essential oils have also been investigated due to their potential to inhibit pathogenic bacteria. Research on the efficacy of all of these feed additives in enhancing animal performance has been inconclusive to date, although it is probably unreasonable to expect any one additive to replace antimicrobial agents in feed (Allen et al., 2013).

Infectious disease in food-producing animals results in significant morbidity, mortality, and economic losses during production. It may also pose a threat to public health and food safety through the transfer of these infectious agents along the food production chain. Microbiome research has been used to better understand the relationship between healthy and disease states in animals. For example, researchers have characterized the gut microbiota of pigs in response to Salmonella Typhimurium (Bearson et al., 2013) and enterotoxigenic Escherichia coli (Xu et al., 2014) challenge, of feedlot cattle colonized with E. coli O157:H7 (Zaheer et al., 2017), and of chickens colonized with Campylobacter jejuni (Han et al., 2017). The nasopharyngeal microbiomes of cattle and swine have also been described in relation to bovine respiratory disease (Gaeta et al., 2017) and Glässer's disease (Correa-Fiz et al., 2016), respectively. Studies have also attempted to associate feed efficiency with the rumen microbiome in beef cattle (Myer et al., 2015) and ileal, cecal, and fecal microbiome in swine (McCormack et al., 2017).

Direction of microbiome research in livestock

Microbiome research in the livestock and agricultural sector is expected to grow as costs for metagenomic sequencing continue to decrease and new associations between the microbiome and animal health become apparent. Integrating new technologies into microbiome projects such metatranscriptomics (transcribed mRNA), metabolomics (metabolites), and metaproteomics (proteins), along with animal data, will greatly expand our understanding of the microbiome-host interactions. In the future, the goal will be to use this information to manipulate the microbiome to enhance performance, food safety, carcass traits, and prevent disease in livestock.

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