

# The Role of Probiotics in Veterinary Medicine



The use of probiotics in veterinary medicine has gained popularity in recent years and many different conditions are now successfully managed with probiotic therapy. Intestinal microflora and the impact of probiotics is currently an area of interest for researchers and clinicians alike. This article will examine the variations in the microflora seen in specific conditions and how probiotics can influence this, with particular focus on the evidence available in dogs and cats.

## The Gastrointestinal Microflora

The gastrointestinal (GI) tract is home to a complex ecosystem of micro-organisms known as the microflora. The microflora consists of bacteria, protozoa, fungi, viruses and archaea and is thought to number around 10 trillion ( $10^{12}$ ) micro-organisms. The micro-organisms can be classified as:

- **Mutualists** - two organisms of different species exist in a relationship in which each individual benefits
- **Commensals** - relationship between two organisms where one organism benefits without affecting the other
- **Parasites** - non-mutual relationship between organisms where one organism, the parasite, benefits at the expense of the other, the host

At birth, the GI tract is initially sterile but is rapidly colonised within hours by bacteria present in the birth canal and surrounding environment. After 24 hours, aerobic and anaerobic bacterial counts exceed  $10^8$  colony forming units (CFU) per gram of intestinal contents<sup>1</sup>. The composition of the microflora is influenced by host genetics and environment, with individuals having very unique intestinal ecosystems which generally remain stable over time. The microflora are resilient to change but can be modulated by diet, stress or antibiotics. Once the inciting cause has been resolved, the microflora will revert to the pre-insult state. The presence of bacteria in early life is important for the development of oral tolerance to commensal bacteria and food antigens, in order to prevent onset of inappropriate immune responses, which are thought to play a key role in the development of chronic intestinal disease<sup>2</sup>.

The GI microflora plays a crucial role in the health of the host. They act as a defending barrier against invading pathogens, aid in digestion and energy harvest from the diet, provide nutritional support for enterocytes, and stimulate the development of the immune system<sup>3</sup>. The resident microflora can protect the host from invading pathogens via the mechanism of 'colonisation resistance.' This involves a competitive state where microbes compete for oxygen, nutrients and mucosal adhesion sites, in addition to creating a physiologically restrictive environment for non-resident bacterial species. This can be via the production of antimicrobial substances, alterations in the intestinal pH, and production of hydrogen sulphide<sup>4</sup>. The microflora have

the ability to ferment complex carbohydrates that include starch and dietary fibre (cellulose, pectin and fructans) which results in the production of short-chain fatty acids (SCFAs). SCFAs provide energy for bacterial metabolism but also for epithelial cell growth<sup>5</sup>. Physiological levels of SCFAs also stimulate intestinal motility, emphasising the importance of microbial fermentation products on host health<sup>6</sup>. Further evidence for the benefits of the intestinal microflora can be exhibited when studying germ-free animals. Dogs raised germ-free have thinner intestinal villi, along with a reduction in the lamina propria and mucosal surface area<sup>7</sup>. They exhibit an underdeveloped lymphoid system and have decreased immunoglobulin concentrations<sup>8</sup>.

It is now known that altered microflora play a role in various GI disorders. The term 'dysbiosis' is used to describe imbalances in the intestinal microflora and can be due to an overgrowth of specific pathogens (e.g. *Salmonella*, *E. coli*, *C. perfringens*) or a generalised reduction in bacterial species richness. For example, studies have shown that there is a reduced species richness seen in dogs and cats with inflammatory bowel disease (IBD) – often there are depleted numbers of bacteroides<sup>9</sup>. Also, dogs and cats with idiopathic small intestinal IBD appear to have a higher proportion of Enterobacteriaceae, such as *E. coli*, compared with controls<sup>(10, 11)</sup>.

Intestinal dysbiosis is associated with a disruption in the GI homeostasis and external manifestations can include diarrhoea, vomiting, weight loss, depression, lethargy, flatulence, borborygmi, changes in appetite and abdominal pain. Factors that may lead to the development of intestinal dysbiosis include:

- Gastrointestinal disease – any involvement of pathogens or disruption of normal motility and digestion results in a dysbiosis developing.
- Antibiotic usage – indiscriminate elimination of intestinal bacteria affects both beneficial and non-beneficial populations.
- Dietary change – can include periods of fasting, altered composition, dietary intolerances and indiscretion (scavenging).
- Stress – cortisol release causes an increase in intestinal pH which favours the growth of potentially pathogenic bacteria (e.g. *E. coli*, *Salmonella*).
- Life-stage – significant changes occur at weaning and into old age.

Such changes in the small intestinal microbiota may lead to various mechanisms that negatively impact the function of the GI tract. Examples include the dehydroxylation of fatty acids leading to impaired fat absorption, alterations in the intestinal barrier with increased intestinal permeability, the

destruction of brush border enzymes and epithelial carrier proteins, and competition for substrates leading to nutrient and vitamin malabsorption<sup>12, 13, 14</sup>.

### Introduction to Probiotics

Probiotics are defined by the World Health Organisation (WHO) as “live micro-organisms which when administered in adequate amounts confer a health benefit on the host.” Probiotics can be used to manipulate the microflora to a more favourable balance. This is especially useful when there is some form of dysbiosis within the GI tract. By providing an influx of probiotics, a normalisation of the microflora may be achieved. There are several ways in which probiotics exert their beneficial effects:

- Interaction with other GI micro-organisms
  - Competition for adhesion sites and luminal substrates
  - Synthesis and release of antimicrobials
    - Production of acids (e.g. lactic acid) which act to decrease luminal pH
    - Production of inhibitory peptides and bacteriocins
- Nutritional
  - Role in the fermentation of complex carbohydrates to short-chain fatty acids
  - Synthesis of vitamins B and K
- Regulation of intestinal epithelial function
  - Increased production of mucus
  - Protection of tight junctions and enhancement of epithelial repair
  - Increase brush border membrane enzyme activity
- Immune effects
  - Activation and modulation of specific and adaptive immune responses

### Probiotics in Veterinary Medicine

The European Food Standards Agency (EFSA) register of approved feed additives lists probiotic species authorised for use in certain animal species. One of two registered strains for use in dogs and cats is the bacteria, *Enterococcus faecium* (NCIMB 10415) 4b1707, which has been approved according to the Commission Regulation (EC) 2015/1053 on the 1st July 2015 as an “Additive for the stabilisation of the intestinal flora.”<sup>15</sup>

The effects of *Enterococcus faecium* (NCIMB 10415) on the faecal microflora were determined in an eight-week cross-over study involving eight dogs (four males and four females, 1.5 years old, 8.8 - 11.5 kg). The animals were kept separate from one another and divided in two homogeneous groups. The control group was given a commercial diet (80 % moisture, crude protein 6 %, crude fibre 0.1 %, crude fat 4.5 %) and the treated group was given this diet supplemented with  $4.5 \times 10^9$  cfu *Enterococcus faecium* (liquid form) per kg feed (certificate of analysis provided). Microbial enumeration of *Enterococci*, *Escherichia coli* and *Clostridium perfringens* were performed on faeces samples collected at the end of each two-week test period. Statistical evaluation was performed using the Latin square design variance analysis (F-Test). In animals receiving the supplemented diet, *E. coli* level was significantly lower than in control animals (decrease of  $8 \times$

$10^9$ cfu/g faeces). *Enterococci* and *Clostridium perfringens* counts in faeces were similar for both the control diet and the supplemented diet ( $9.1 \times 10^{10}$ cfu/g faeces).<sup>15</sup>

A field study was carried out in order to investigate the effects of *Enterococcus faecium* (NCIMB 10415) on faecal counts of *Salmonella* spp., *Campylobacter* spp. and *Clostridium* spp. in healthy dogs (based on counts made using selective media without confirmatory studies). Twelve dogs from private households (aged from 1 to 12 years) belonging to different races (2.5 kg live weight (LW) to 70 kg LW) were used. The animals were fed twice daily with a commercial diet (crude protein 26 %) according to their physiological needs. For an 18-day period, *Enterococcus faecium* (NCIMB 10415) was added daily to the diet at a concentration of  $9.2 \times 10^9$ cfu per dog (a certificate analysis was provided). *Salmonella* spp., *Campylobacter* spp. and *Clostridium* spp. counts in faeces were measured prior to the probiotic administration and after the 18-day experimental period. Rectal sampling was carried out in order to avoid any faeces contamination. Statistical evaluation was performed using t-test. The results of this field study showed that the *Salmonella* spp. (highest observed concentrations:  $1.5 \times 10^4$ cfu/g) and *Campylobacter* spp. (highest observed concentrations:  $4 \times 10^5$ cfu/g) counts in faeces were not affected by a daily ingestion of *Enterococcus faecium* (NCIMB 10415). However, *Clostridium* spp. (highest observed concentrations:  $8 \times 10^4$ cfu/g) counts in faeces were found to be significantly lower after the probiotic administration.<sup>15</sup>

*Benyacoub et al.*<sup>16</sup> investigated the capacity of the probiotic, *Enterococcus faecium* (NCIMB 10415) to stimulate immune functions in young dogs. Puppies were allotted to two groups, receiving either a control diet or a diet supplemented with  $5 \times 10^8$  cfu/day of probiotic *Enterococcus faecium* (NCIMB 10415) from weaning to one year of age. Faecal and blood samples were collected from the dogs at different time points for the measurement of faecal immunoglobulin (Ig) A, circulating IgG and IgA, and the proportions of lymphoid cell subsets. Faecal IgA and canine distemper virus (CDV) vaccine-specific circulating IgG and IgA were higher in the group receiving the probiotic than in controls. There were no differences in the percentages of CD4+ and CD8+ T cells between the groups, but the proportion of mature B cells [CD21/major histocompatibility complex (MHC) class II] was greater in those fed the probiotic. This study demonstrated *Enterococcus faecium* enhanced specific immune functions in young dogs, thus offering new opportunities for the utilisation of probiotics in canine nutrition.

The effect of the probiotic, *Enterococcus faecium* (NCIMB 10415), on diarrhoea in dogs housed in an animal shelter was investigated by *Bybee et al.*<sup>17</sup>. This double-blinded and placebo-controlled study housed 182 shelter dogs in two separate rooms. For four weeks, animals in one room were fed *Enterococcus faecium* while animals in the other room were fed a placebo. After a one-week washout period, the treatments by room were switched and the study continued an additional four weeks. A standardised faecal score system was applied to faeces from each animal every day by a blinded

individual. Faeces of animals with and without diarrhoea were evaluated for enteric parasites. Data were analysed by a generalised linear mixed model using a binomial distribution with treatment being a fixed effect and the room being a random effect. Overall, 9.8% (10 of 102) in the probiotic group had at least one episode of diarrhoea and 12.5% (10 of 80) of dogs in the placebo group had at least one episode of diarrhoea.

Vahjen and Mannen<sup>18</sup> investigated the effect of *Enterococcus faecium* on bacteriological counts of *Salmonella* spp., *Campylobacter* spp. and *Clostridium* spp. in faeces of healthy dogs under different feed and environmental conditions. For the study, 12 dogs kept in households were used for an 18-day supplementation with a patented commercially available strain of *Enterococcus faecium* (NCIMB 10415). The probiotic was administered orally at a dose of  $9.2 \times 10^9$  CFU/dog. The faeces were collected before the beginning of the supplementation and at the end of the 18-day application period. In order to exclude contamination, all faeces were taken rectally. Before and at the end of the experimental period, total *Salmonella* spp., *Campylobacter* spp. and *Clostridium* spp. counts were determined in fresh faeces using selective media. It was demonstrated that the 18-day application of the probiotic *Enterococcus faecium* induced modifications on the gastrointestinal microflora in all dogs. *Clostridium* spp. counts were significantly reduced in 10 of 12 dogs.

#### Introduction to Prebiotics

Prebiotics are defined as a “selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health”<sup>19</sup>. They are a food source preferentially chosen by the beneficial bacteria

that inhabit the gastrointestinal tract such as Lactobacilli and Bifidobacteria. The International Scientific Association for Probiotics and Prebiotics (ISAPP) currently defines three criteria for a good prebiotic<sup>20</sup>:

- Resistant to degradation by stomach acid, mammalian enzymes and hydrolysis
- Fermented by the intestinal microbes
- Selectively stimulates the growth and/or activity of beneficial micro-organisms in the gut

The fermentation of prebiotics by the intestinal bacteria results in the production of short-chain fatty acids such as acetate, propionate and butyrate, whilst also encouraging the growth of certain bacterial species. It has also been suggested that prebiotics can play a direct role in host defences and immunomodulation. One study reported that short-chain fatty acids are able to increase mucin production and decrease bacterial translocation by binding to immune cells within the gut-associated lymphoid tissue. Energy balance within the gut can also be fine-tuned by the gut flora, with the short-chain fatty acid butyrate being a direct energy source for colonocytes. This positive benefit of prebiotics has been cited as having potential for treating human inflammatory bowel conditions, in helping to restore metabolic function of the cells and accelerating repair of the intestinal wall by helping mucosal integrity. They have also been shown to contribute to a direct down-regulation of pro-inflammatory cytokines.

#### Prebiotics in Veterinary Medicine

Swanson *et al.*<sup>21</sup> investigated the effects of FOS on the concentration of gut microbial populations, fermentative end products and nutrient digestibility in healthy adult dogs. The addition of FOS resulted in lower ( $P=0.08$ ) *Clostridium perfringens* and greater faecal butyrate ( $P=0.06$ ) and lactate ( $P$



< 0.05) concentrations. FOS was also found to increase faecal levels of Bifidobacteria ( $P < 0.05$ ) and Lactobacilli ( $P = 0.08$ ) while reducing the levels of faecal ammonia, isobutyrate, isovalerate and total branch-chain fatty acid concentration ( $P < 0.05$ ). These findings suggest FOS positively influences indices of gut health.

The effect of FOS on dogs suffering from small intestinal bacterial overgrowth was investigated by Willard *et al.*<sup>22</sup> They studied sixteen IgA-deficient German Shepherd dogs with small intestinal bacterial overgrowth; half were given a standard chicken-based diet while the other half were fed the same diet supplemented with FOS. After being fed the diets for 46-51 days, the FOS-supplemented animals had significantly ( $P = 0.04$ ) fewer aerobic/facultative anaerobic bacterial colony forming units in fluid from the duodenum/proximal part of the jejunum, as well as in the duodenal mucosa.

The effect of FOS on levels of immunoglobulin in the colostrum and milk of bitches was investigated<sup>23</sup>. Bitches that were supplemented with FOS exhibited higher colostrum and milk immunoglobulin (Ig) M concentrations. It was also noted that supplemented bitches exhibited a greater immune response to vaccination. Intranasally immunised puppies exhibited a higher *Bordetella bronchiseptica*-specific IgM response.

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