Prebiotics, probiotics and faecal transplants in cats: where are we now?

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Author

Prof. Silke Salavati, Dr.med.vet., PhD, Dipl.ECVIM-CA, FHEA, FRCVS

Personal Chair in Small Animal Gastroenterology

Hospital for Small Animals, Royal (Dick) School of Veterinary Medicine and The Roslin Institute, College of Medicine and Veterinary Medicine, The University of Edinburgh

Easter Bush Campus, Midlothian, EH25 9RG, United Kingdom

Email: Silke.Salavati@ed.ac.uk

Abstract

Chronic gastrointestinal signs in cats can be a frustrating problem, especially as there is little evidence for best diagnostic or therapeutic approaches specific to cats, which are mostly extrapolated from dogs. There is often an additional concern that insidious infectious agents have been missed, or that chronic intestinal inflammation could transition to a neoplasm. Empirical use of antimicrobials is problematic both from a global health perspective and due to the mounting evidence of the disruptive effect of commonly used antibiotics on the feline intestinal microbiota. Antibiotic-induced dysbiosis is inevitable and recovery of the microbiota after cessation of antibiotic treatment cannot be expected in all cats. Due to an increasing awareness of these challenges amongst veterinary professionals, "novel" or "alternative" treatment approaches focusing on manipulation and improvement of the intestinal microbiota are sought after. This includes the use of prebiotics, probiotics or synbiotics, dietary interventions inducing production of beneficial postbiotics as well as faecal microbiota transplantation (FMT). This article explores the evidence for the use of these potential treatments, the concept of dysbiosis, and details novel discoveries in the context of the feline microbiota.

Manuscript:

Both acute and chronic diarrhoea are common presenting complaints in cats in primary care practice. While acute diarrhoea is often self-limiting and might only require supportive care, there is still a perceived or real pressure for the attending veterinarian to "do something" to shorten the duration of clinical signs. The more than anecdotal knee-jerk grab for a quick injection of antibiotics and "anti-inflammatory" (often glucocorticoids) is still far too common in many instances, but there is an increasing awareness that neither is indicated nor "harmless", and alternative treatments – ideally evidence-based but at the very least non-harmful – are sought by more and more veterinarians.

Pull quote: **"A knee-jerk grab for a quick injection of antibiotics and glucocorticoids is still far too common as a standard practice when treating acute diarrhoea"**

Chronic gastrointestinal (GI) signs in cats can be a frustrating problem, especially as there is little evidence for best diagnostic or therapeutic approaches specific to cats, which are mostly extrapolated from dogs. There is limited availability or accuracy of gut-specific biomarkers or other predictors of aetiological diagnoses or treatment successes, often leading to a long list of necessary tests or empirical treatment trials. Diagnostic workup often involves a large number of often unrewarding tests to rule out "other" conditions than idiopathic inflammation along the lines of chronic enteropathy (CE) or inflammatory bowel disease (IBD). While useful from a diagnostic standpoint to "know what it is not", this long list of rule-outs can become even more frustrating for cat owners, as it is time- consuming, might involve perceived or true stress to the cat due to multiple vet visits, and can cause financial concerns. Similarly, keeping owner (and cat) compliance with a succession of food trials can be hard work with the pressure for a "quick fix" mounting. In many cases achieving success with an appropriately performed food trial requires specific attention and support from veterinary professionals (e.g. specifically trained nurses), without which they are doomed to fail, further reducing the chances of managing these chronic GI cases successfully.

Pull quote: **"The success of an elimination food trial stands and falls with the availability of specific and timely support from veterinary professionals like nutrition-trained RVNs"**

Specific to cats with chronic GI signs, there is also more often than not the additional concern that an insidious infectious agent (for example *Tritrichomonas blagburni* [formerly *T. foetus*]) has been missed, or that if chronic intestinal inflammation remains unchecked for too long, transition to a neoplastic condition (e.g. small cell/ low grade lymphoma) is a real possibility.

Again, while there is an increasing awareness of potential harm that antibiotics can do to the already dysbiotic intestinal microbiota communities in cats with CE/IBD (as they can to healthy ones), and that glucocorticoids can either lose efficacy over time or create unwanted adverse effects despite cats being more resistant to those than dogs, evidence for suitable alternative treatments is largely lacking.

In a referral setting, we seem to mostly see cats with chronic GI signs that belong to one of 2 larger groups with separate demographics/ signalement and final diagnoses: either young cats (often pedigree) with a typical history of having had large or mixed bowel diarrhoea "since in the owners possession", e.g. likely from weaning onwards, but are typically otherwise well, or a group of more middle-aged to older cats that fall more in line with the "typical" perception of CE/IBD, and can behave similar to dogs with this condition (e.g. small bowel diarrhoea, waxing and waning additional clinical signs like weight loss, inappetence, thickened guts on abdominal palpation etc.).

Especially in that group of younger cats (often < 1 year old) with very long standing diarrhoea, a repeated search for infectious agents is advocated, as the most persistent ones like *T. blagburni* can be intermittently shed. Here, sampling technique also seems to play an important role: Anecdotally, mucoid faecal samples are said to be preferable (rather than solid faeces), or a sample from mucus around the anus/ the perineal area which can give a good yield if *T. blagburni* is present (for both direct microscopy and PCR). A positive PCR result for *T. blagburni* is also more likely if a so called faecal loop is used, which are even superior to a "colonic flush" sample (1), but the latter might be more achievable in practice (see box 1) and the flush is still superior compared to a spontaneously voided faecal sample.

Box 1: A colonic flush is a technique to obtain a sample for *Tritrichomonas blagburni* PCR. For this, a soft rubber catheter is inserted rectally as far as possible into the colon. This can be done awake or under a mild sedation. A small amount of saline (e.g. 10 ml) is then administered through the catheter and aspirated back (if cat allows can be accompanied by gentle massage of the colon). The fluid obtained is usually about 1/3 or ½ of the instilled volume and should visibly contain some diluted colonic content. The procedure can be repeated if necessary. The colonic flush fluid can be assessed directly under the microscope, but PCR is a more sensitive method to detect *T. blagburni*.

In the older group of cats with chronic GI signs, the approach is often similar to dogs (2): ruling out extra-GI diseases (with chronic kidney disease, hyperthyroidism, pancreatic diseases and hepatobiliary diseases top of the list), checking for cobalamin deficiency, and documenting intestinal inflammation while ruling out as much as possible the presence of neoplasia (see box 2). This is then often followed by empirical treatment approaches. It is said that cats with food-responsive chronic enteropathy (FRE) are more likely to positively respond to a diet change quickly (2-3 weeks) than dogs with FRE, but published evidence for this is scarce (3,4).

Box 2. List of differential diagnoses and associated diagnostic tests to perform in cats with chronic GI signs

	Differential diagnosis	Suggested initial diagnostic test
Extra-gastrointestinal	Chronic kidney disease	Urinalysis, serum creatinine, serum SDMA, systolic blood pressure
	Hepatobiliary conditions	Serum albumin, urea, ALT, ALP, GGT, bilirubin; Abdominal ultrasonography (± FNA of abnormal structures; consider cholecystocentesis for cytology and culture); Serology: toxoplasma IgG/ IgM; (consider liver biopsies if performing full thickness gut biopsies)
	Hyperthyroidism	Total thyroxin (TT4)
	Pancreatitis Exocrine pancreatic insufficiency	fPL; (consider pancreatic biopsies if performing full thickness gut biopsies) fTLI, serum cobalamin (Vit B12)
Gastrointestinal	Infectious disease	Routine faecal flotation/ sedimentation + Giardia antigen PCR for *Tritrichomonas blagburni* Consider testing for FCoV shedding by PCR (protracted enteric FCoV infection)
	Inflammatory disease (CE/ IBD)	Abdominal ultrasonography, serum cobalamin (Vit B12); Endoscopic mucosal pinch biopsies (consider full thickness biopsies if muscularis layer of SI wall mostly involved, or excisional biopsy if obstructive granuloma)
	Neoplastic disease	For solitary masses or enlarged lymph nodes: consider FNAs and/ or (excisional) biopsy For diffuse neoplasms like lymphoma: serum cobalamin (Vit B12), endoscopic mucosal pinch biopsies (consider full thickness biopsies if muscularis layer of SI wall mostly involved)

To some extend it seems “lucky” that antibiotic-responsive chronic enteropathy (ARE) is to be less of a feature in cats than in dogs (4), however, the author suggests some of this lack of response might be due to the historical problems around palatability of drugs like metronidazole in cats, which - alongside tylosin - is the most commonly used antibiotic in CE. While this might also explain why there is no study on the effect of metronidazole or tylosin on the microbiota specifically in cats, it is likely that the effect of this antimicrobial is as devastating to the feline microbiota than it is in dogs (5), an effect that has similarly seen with tylosin (6). There is however, evidence of the disruptive effect of other commonly used antibiotics like amoxicillin-clavulanic acid (7,8), doxycycline (7) and clindamycin (9) in cats, that demonstrate intestinal dysbiosis is inevitably induced, cannot be prevented or ameliorated by the addition of synbiotics and recovery of the microbiota to baseline is not always a given (figure 1).

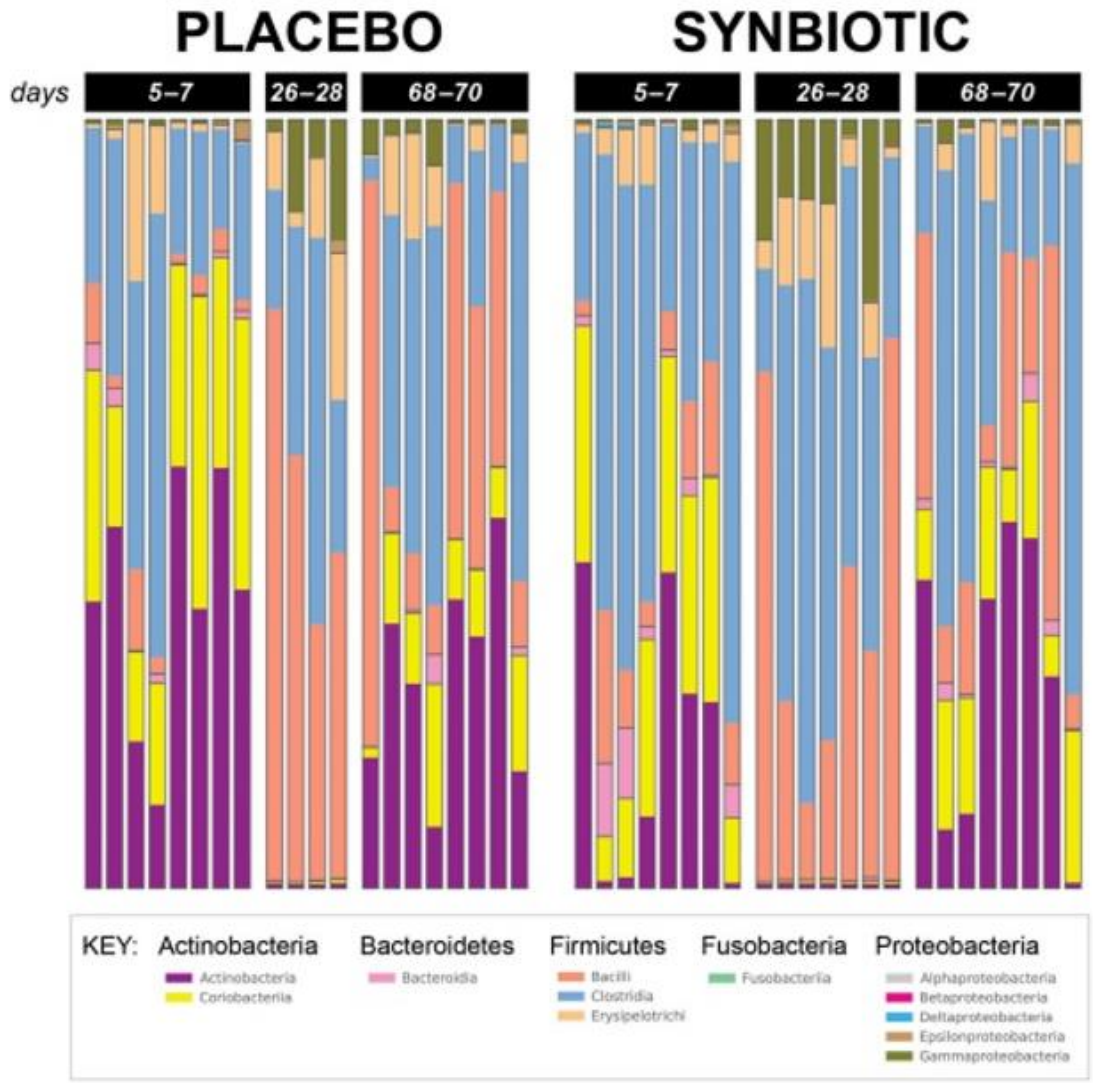

Figure 1. Phylum- and class-level composition of feline faecal microbiota. Each column represents the microbiota of an individual cat. 16 healthy research cats received clindamycin for 21 days with either placebo or synbiotic (Proviable-DC®, Nutramax). Faecal samples were collected during baseline (days

5-7), antibiotic administration (days 26-28) and 6 weeks after antibiotic discontinuation (days 68-70). The synbiotic was unable to prevent significant alterations of microbiota alpha and beta diversity, and return to baseline was variable. From Whittemore et al. 2019 (9).

There is the increasing concern that antibiotics could on one hand set individual cats up for an increased risk of developing chronic inflammation of the gut mucosa later in life (as demonstrated now for people (10) and suspected in dogs), or on the other hand perpetuate dysbiosis in cats with CE/IBD that appear "responsive" to antibiotics on the surface (e.g. a clinical response does not necessarily mean true treatment of the underlying condition, but rather acts as a smoke screen), as has been demonstrated in dogs (11).

Pull quote: **"There is an increasing concern that antibiotics could set up individual cats for an increased risk of developing chronic gut inflammation or perpetuate dysbiosis in cats that already suffer from such conditions like inflammatory bowel disease"**

Due to all of the above challenges, an increasing desire to use antibiotics more evidence-based (12), together with an increasing understanding of the involvement of the complex GI microbiota community in many of the acute and chronic GI conditions seen in cats, "novel" or "alternative" treatment approaches – mostly focusing on manipulation or improvement of the microbiota – are sought after (box 3). These are namely prebiotics, probiotics or a combination of them (synbiotics), as well as more sophisticated dietary interventions (and their potential to direct the production of so called postbiotics through specific fibre blends), or even faecal microbiota transplantation (FMT).

Box 3. Definitions of…

… probiotics: live organisms that confer a health benefit onto the host when administered in adequate amounts

… prebiotics: complex carbohydrates that promote the growth or function of probiotics (often dietary fibres)

… synbiotics: a preparation containing both pre- and probiotics

… postbiotics: bacterial metabolic byproducts that have a benefit to the host organism (example: short chain fatty acids like butyrate or plant-derived polyphenols)

At the moment, evidence for many of these new treatment options are lacking while health claims of products are ubiquitous, which is facilitated by the unprotected use of terms such as "probiotic" (13). Some of the paucity of information specific to cats is likely due to the fact that the understanding of the composition, diversity and function of the feline intestinal microbiota is much less complete than in dogs and people, and that techniques to investigate

the microbiome are still relatively expensive (e.g. next generation sequencing) and hence largely reserved for research purposes.

However, there is some evidence for the use of specific single- or multiple-strain pro- or synbiotics in certain conditions in cats. Probiotics theoretical benefits within the GI tract include the displacement of intestinal pathogens, for example by competing for binding sites on intestinal epithelial cells, the stimulation of mucus and antimicrobial peptide production, the up-regulation of various non-specific cellular defence mechanisms and interaction with immune cells within the intestinal lamina propria with subsequent "anti-inflammatory signalling" via cytokines and chemokines (13). Many of these actions are likely strain specific, as even bacterial strains of the same species have been shown to elicit a different cellular response (for example different Lactobacilli can be pro- or anti-inflammatory), and more details can be found in a recent review (13).

Enterococcus faecium (EF) is one of the most widely used probiotics in small animals, and a small number of studies are available where EF has been used alone or in combination with other probiotic strains in the treatment of both acute and chronic conditions in cats (table 1). From these studies, it can be concluded that there was no effect of EF on shelter-(stress? parasite?) associated diarrhoea in cats (14), no benefit in giving it alongside antibiotics like amoxicillin (8), but that it can be used as an adjunctive treatment for Tritrichomoniasis (15), as it seems to prevent relapses (although it is unclear if any EF preparation is suitable or if the specific synbiotic used in this study is needed). A commercially available lactic acid bacteria (LAB) mixture (also containing EF) was unable to make a large impact when given alongside clindamycin to healthy cats (9).

Pull quote: **"There is benefit in giving an *Enterococcus faecium* containing synbiotic alongside ronidazole in cats with *Tritrichomonas blagburni* infection."**

In another study, oral administration of a probiotic *Enterococcus hirae* mitigated the increase in intestinal permeability and faecal water loss resulting from *E.coli* infection in purpose-bred kittens, as well as reduced shelter-associated diarrhoea in kittens, so could be a viable choice for this demographic and purpose, but this is not currently commercially available (16). A commercial probiotic LAB cocktail (called SLAB51^{TM}) containing several lactobacilli, bifidobacteria and a streptococcus has been used in a single study in cats with constipation or idiopathic megacolon with some success (17), so this can be an acceptable adjunctive treatment in these conditions. However, there was no control treatment with other frequently used measures like for example a high fibre diet. Recently, some more unusual probiotics have been assessed in small clinical trials in cats: *Bacillus licheniformis*-fermented products (BLFP), previously used as food additives in poultry and pigs, have shown to improve faecal consistency in a small number of cats with otherwise uncharacterised diarrhoea (18). A probiotic preparation containing *Saccharomyces boulardii* and *Pediococcus acidilactici* improved some faecal characteristics in healthy cats (increased butyrate content, reduced inflammatory markers like calprotectin, increased antioxidant capacity), but did not alter their microbiota composition or diversity significantly (19).

Table 1. Pro- and synbiotics assessed in cats. cfu = colony-forming units, CI = confidence interval, IQR = interquartile range, EF = *Enterococcus (E.) faecium*. FHV-1 = feline herpesvirus 1, FOS = fructo-oligosaccharides, ICC = interstitial cells of Cajal, IEC = intestinal epithelial cells, LAB = lactic acid bacteria, OUT = observed taxonomic units, TF = *Tritrichomonas foetus*.

Pro- or synbiotic used (trade name, manufacturer)	**Dose**	**Animals (n)**	**Disorder**	**Observed outcomes**	**Reference**
***E. faecium* SF68 NCIMB 10415** (Fortiflora®, Nestle Purina)	2.1 x 10^9 cfu/ day	217 (130 treated with EF, 87 treated with placebo)	Shelter-associated diarrhoea (some cats also positive for various parasites)	• No difference in prevalence or duration of diarrhoea (26% with EF, 32% with placebo)	Bybee et al., 2011 (14)
***E. faecium* SF68 NCIMB 10415** (Commercial product not specified)	Not disclosed	27 (13 EF, 14 placebo)	Antibiotic-induced diarrhoea (potentiated amoxicillin)	• No statistical difference in faecal scores between treatment groups • No difference in number of observed species of the faecal microbiota between treatment groups	Torres-Henderson et al., 2017 (8)
***E. faecium* DSM 10663 NCIMB 10415 4b/E1707** + FOS and gum Arabic, kaolin, montmorillonite, pectin, alpha-glucan butyrogenic, patented mucopolysaccharide starch (Pro-Kolin® paste, Protexin Ltd.)	Dose not disclosed	26 (13 ronidazole 10-30 mg/kg q24h & placebo; 13 ronidazole 10-30 mg/ kg q24h & synbiotic)	Natural *Tritrichomonas foetus/ blagburni* infection	• In both groups faecal scores and body weight improved • Cats on synbiotic had significantly less relapses (2/13) compared to placebo treated cats (8/13)	Lalor et al., 2012 (15)
Enterococcus hirae strain 1002-2 (isolated from a healthy kitten ileal mucosa)	1 x 10^8 cfu/day, given until body weight of > 2 lb was reached (median	Healthy kittens < 12 weeks of age admitted to a shelter, 58 randomised to probiotic group,	Shelter-associated diarrhoea (some cats might also have had parasites, but they all received same	• Kittens with the probiotic were 3.4 times less likely to develop diarrhoea compared to the placebo group (OR = 0.294, 95% CI 0.109-0.792, p = 0022)	Gookin et al., 2022 (16)

	number of days in study was 17, IQR 14-26)	72 randomised to placebo group	antiparasitic regimen)	• There was no difference in prevalence of faecal infectious agents in both groups at beginning or end of study • No difference in microbiota diversity (Shannon index, number of OTUs) or phylogenetic composition (beta diversity) between groups • Kittens receiving probiotics had a higher relative abundance of Enterococcus and ower abundance o Megamonas	
<u>LAB mixture:</u> ***B. bifidum,*** ***E. faecium,*** ***E. thermophilus,*** ***L. acidophilus,*** ***L. bulgaricus,*** ***L. casei,*** ***L. plantarum*** + FOS and arabinogalactan (Proviable-DC®, Nutramax Ltd.)	1 x 10^{10} cfu/ day	16 (8 treatment, 8 placebo)	Antibiotic-induced diarrhoea (clindamycin)	• Higher abundance of faecal Ruminococcaceae in probiotic-treated group • No difference of dysbiosis index, microbiota richness or diversity indices between treatment groups • Some complex metabolomic changes were observed, but most likely due to antibiotics rather than probiotics	Whittemore et al., 2019 (9)
<u>SLAB51 blend:</u> ***Scc. thermophilus* DSM32245,** ***L. acidophilus* DSM32241,** ***L. plantarum* DSM32244,** ***L. casei* DSM32243,** ***L. helveticus,* DSM32242,** ***L. brevis* DSM27961,** ***B. lactis* DSM32246,**	2×10^{11} cfu lyophilised bacteria per 5 kg body weight	10 with naturally occurring disease (10 healthy cats used as controls for tissue, but not treated with probiotic)	Chronic constipation (7/10)/ idiopathic megacolon (3/10)	• Clinical severity score significantly reduced at end of probiotic treatment • Number of ICC increased significantly • Significant increase in faecal streptococci and lactobacilli after treatment (of total of 9 analysed taxa)	Rossi et al., 2018 (17)

***B. lactis* DSM32247** (SivoMixx®, Mendes/ Omendes)					
Saccharomyces boulardii, Pediococcus acidilactici	2×10^{10} cfu/g of *S. boulardii* 2.5×10^{10} cfu/g of *P. acidilactici*, mixed in a 1:1 ratio. Of this, given 0.5 g/kg body weight	10 (5 in treatment group, 5 in control group)	Healthy cats	• Different microbiome structure, with 66 OTUs unique to the control group, 81 OTUs unique to the probiotic group, and 369 shared OTUs • No significant difference in relative	Li et al., 2023 (19)
E. faecium SF68	5×10^8 cfu/day	12 (6 EF, 6 placebo)	FHV-1 infection	• Cats in EF group had less days of conjunctivitis (16%) vs. cats in placebo group (30%), but only 1 cat in the EF group had significantly less days of conjunctivitis compared to the 30 day period prior to supplementation • No statistical difference in FHV-1 shedding (per PCR) • All cats had high FHV-1 antibody titres, with no change throughout study • Lymphocytes from all cats responded to stimulation with FHV-1 antigens in vitro, with no difference between groups	Lappin et al., 2009 (20)

There is very little evidence for the use of probiotics outside the GI tract in cats. One study assessed the use of EF in latent feline herpesvirus-1 (FHV-1) infection (see table 1), and while the authors conclude that the probiotic supplement might have decreased morbidity in the infected cats, this was only noticeable in 2 cats, with the percentage of days with conjunctivitis, and number of cats PCR positive for FHV-1 throughout the study was not different between the EF treated and the placebo group (20). A recent study investigated the effect of an *E.coli* (Nissle 1917) probiotic on the *in vitro* growth of feline uropathogenic *E.coli* (UPEC) isolates, and found that Nissle 1917 adversely affected the growth of 82.5% of all isolates and 100% of the multi-drug resistant UPEC strains (21). The clinical utility of these findings or sensible mode of delivery in clinical cases with UPEC urinary tract infections has not been established.

The evidence surrounding prebiotics and dietary fibre (DF) in cats is challenging to evaluate. While the physiological facts suggest that cats are able to ferment a broad range of DF, little research has been done on these in the context of specific diseases. Most studies assessing the effect of prebiotics in cats are feeding trials investigating nutrient digestibility or changes in fermentation properties (13). Particularly fructo-oligosaccharides (FOS) were originally described as useful fibre source for promoting feline GI health based on changes in the microbiota, but in a number of studies there were also no beneficial effects demonstrated, which might have been a dose-effect (22). There are no studies investigating the effect of specific DFs or prebiotics alone (ie. not as a synbiotic) in cats with GI conditions apart from one exception: a psyllium-enriched diet was found to be efficient in the management of recurrent constipation, but this study had no control group (23).

Pull quote: **"Fructo-oligosaccharides and psyllium are prebiotics that might be able to promote feline gut health, but evidence for fibre use in cats with specific conditions is generally lacking."**

The production of targeted postbiotics might be achieved by selectively increasing specific pre-, pro- or synbiotic components in the diet. For example, the short chain fatty acid (SCFA) acetate is produced by many different bacteria, but propionate and butyrate are produced by specific bacteria only. Postbiotics are proposed to either act locally in the colon or could be taken up by the host into the systemic circulation (22,24). The most important factor that determines which postbiotics are being formed is the amount and type of substrate available for microbial digestion. Providing carbohydrates to the gut microbiota results in saccharolytic fermentation, which increases production of SCFAs. SCFAs reduce inflammation, maintain mucosal integrity and repair, and stimulate feline colonic smooth muscle contractions. Another source of postbiotics that has recently become more of a focus of interest, are polyphenols (25). They are a diverse class of plant metabolites, and a health-promoting effect has been suggested in cats via the modulation of the microbiota and reduction of inflammatory markers. The author has had some good success in treating young cats with long-standing large bowel diarrhoea (the group of cats described above that often also could

have insidious infections) with a novel commercial diet containing specific (proprietary) fibre blends including polyphenols (Hill's Biome).

While some of the novel information on pre-, pro- and postbiotics is promising, FMT - defined as the transfer of the entire intestinal microbial community from a healthy donor to a dysbiotic recipient with the goal to modulate the recipients microbiota composition and function - is considered an extremely novel and "cutting edge" treatment approach. So much so, that practitioners are mostly unaware of FMT as a potential treatment options, and experience with it is limited (26). Indications, methods of application, and appropriate donor selection criteria are largely unknown and unstandardised, making it harder to give recommendations of its use beyond anecdotal evidence and "expert opinion". To appreciate the potential benefits of FMT over probiotics, one has to understand the concept of dysbiosis. Dysbiosis is an ill-defined disruption of intestinal homeostasis, which in itself is driven by a number of factors. In people this includes host genetics, environmental triggers, antibiotics and other medications, dietetic choices, lifestyle, hygiene and many more. Dysbiosis can be categorised into 3 types (see box 4; (27)), but these are not easy to routinely characterise in cats beyond the commercially available dysbiosis index (DI). In addition, the current definition of dysbiosis does not account for a loss or disruption of particular microbiota function.

Box 4. Types of dysbiosis. Modified from Talapko et al. 2022 (27)

No	Dysbiosis type
1	Loss of beneficial bacteria
2	Overgrowth of potentially pathogenic bacteria
3	Loss of overall bacterial diversity

Dysbiosis is likely present in a lot of GI and extra-GI conditions and has so far been documented in cats with acute and chronic diarrhoea (e.g. CE/IBD) (28), intestinal lymphoma (29), Small Intestinal Bacterial Overgrowth (SIBO) and antibiotic-responsive CE (30), *Giardia duodenalis* infection (31), but also in more systemic conditions like obesity (32) and Diabetes mellitus (33), even in Feline Infectious Peritonitis (34). Consequently, FMT, could have the potential to become a primary or ancillary treatment in many different feline diseases. Of note, FMT is likely to be more effective in achieving comprehensive changes of the microbiota than individual strains of probiotics, given that an entire functioning symbiotic ecosystem is transferred, that not only contains bacteria, but also fungi, protozoa, archea, viruses and phages as well as all their metabolic products and byproducts. However, it is not clear if and to what extent FMT will be causing a permanent shift in the recipients microbiota composition, if there is such a thing as "true" colonisation or engraftment of the new flora, or if FMT is more likely to have a transient effect based on "outcrowding" of pathogens or the delivery of metabolites or signalling molecules that promote "self-healing".

Pull quote: **"FMT is the transfer of the entire intestinal symbiotic microbial ecosystem from a healthy donor to a recipient. It contains bacteria, fungi, protozoa, archea, viruses and phages".**

There is also the question of appropriate donor selection and testing, which likely will have nuances depending on the disease treated and geographical differences in pathogen prevalence. In people – where for some acute dysbiotic conditions like recurring *Clostridium difficile* infection (CDI) FMT is considered part of recommended standard treatments in some countries – a dichotomous approach to donor selection requirements is already observed: in most cases of acute dysbiosis, "any" healthy donor will be sufficient, whereas in complex diseases where dysbiosis is more subtly characterised by the absence, presence or imbalance of individual taxa, donor screening is more complicated and likely computational models will be required to "match" a recipient with an ideal donor (35). This likely explains observed outcomes and success rates in people, where FMT in CDI has a success rate of > 97%, whereas in IBD FMT leads to an improvement in approximately 30% of cases.

In dogs, small case series and a small number of randomised controlled trials using FMT are available. FMT has been used in healthy dogs given tylosin to assess recovery of antibiotic-induced dysbiosis (n=6) (36), in unresponsive CDI (n=8) (37), refractory Giardia sp. infection (n=4) (38), acute haemorrhagic diarrhoea syndrome (n=4) (39), parvovirosis (40) and different groups of dogs with chronic inflammatory enteropathies (a total of 67 dogs across 8 studies) (38,41–47), with variable success.

A feline case of a 6-year old domestic short hair cat with chronic vomiting and diarrhoea poorly responsive to diet change, antimicrobials, glucocorticoids and probiotics that eventually responded favourably to a single FMT (administered via enema and endoscopically into the duodenum) was reported in 2013 (41). Another single feline case report describes the use of FMT as a rectal enema to a 10 year old Abyssinian cat with ulcerative colitis that was unresponsive to conventional therapy (48). The patient relapsed 5 weeks post FMT and a second FMT was given. Some improvement was noted 1 month after the second FMT procedure, with full improvement achieved at 3 month and sustained at least until 11 months after the initial presentation.

Recently, a larger study evaluated the use of a commercially available capsuled (freeze-dried) FMT for approximately 25 days in 68 cats with chronic digestive conditions (characterised by vomiting, diarrhoea, constipation, or "other", but otherwise undefined as recruited via social media) (49). Owners collected faecal samples before and 2 weeks after the course of capsule FMT and recorded the faecal consistency. The study aimed to evaluate the effectiveness of FMT capsules by assessing the reduction of clinical signs and described microbiota changes of FMT recipients and their correlation to selected host factors to see if these could be predictors of response to treatment: clinical signs, IBD diagnosis, recent antibiotic use and dietary category. Of the included cats, 77% improved, with 16% experiencing no change and 7% worsening.

Pull quote: **"In a recent study, capsuled FMT administered to nearly 70 cats with clinical signs of diarrhoea, vomiting or constipation resulted in an improvement in 77% of these cats."**

A slightly greater proportion of "responders" were female, fed wet with raw food and experiencing constipation compared to "non-responders", which instead had a greater number of cats with IBD or suspected IBD, slightly greater ratio of cats with vomiting and diarrhoea, and a greater number of cats that ate wet food with dry food. About 50% of cats had recently been given antibiotics in both groups. Microbiota analysis results showed that the faecal microbiota diversity was best predicted by dietary category and clinical signs, with bacterial communities from cats with vomiting/ diarrhoea found to be less even than those of cats with only diarrhoea, constipation or other clinical signs. Interestingly, microbiota richness, evenness and phylogenetic diversity did not vary in pre-FMT vs. post-FMT samples. Cats' individual identities explained 83% of the variance in the microbiome, indicating a strong individual microbiota profile resilience. When examining specific bacterial taxa before and after FMT in some of these cats, three genera (*Blautia, Collinsella, Negativibacillus*) tended to be decreased in cats with vomiting and diarrhoea. In addition, specifically the relative abundance of the genera *Peptococcus* and *Ruminococcus* was slightly increased in non-responder cats; and cats with IBD tended to have an increase in the relative abundance of Helicobacter. After FMT, no complete microbiome engraftment was observed, but approximately 13% of bacterial amplicon sequence variants (ASVs) were shared between donor and recipient on average. The most commonly shared ASVs (engrafted in > 12 of the 68 FMT recipients) were *Bacteroides, Clostridium, Lachnospira, Oscillibacter, Subdoligranulum, Fusobacterium, Blautia*, and a number of unclassified groups. So while the oral capsule FMT is potentially effective in alleviating a range of clinical signs associated with gastrointestinal disease in cats, the recipients' faecal microbiota remained significantly different and distant from their donors, 2 and 8 weeks after cessation of FMT, hence a complete microbiota engraftment is not occurring. While this might be in theory beneficial, the authors of the study suggest that based on their results, engraftment might not be necessary for clinical effectiveness of FMT.

Pull quote: **"Microbiota diversity did not change after FMT and no shift in microbiota composition was observed. This might suggest that engraftment is not necessary for clinical effectiveness of FMT."**

Based on the available information, the author currently recommends feline microbiome modification the following way (compare with figure 2): for acute/ uncomplicated diarrhoea, consider an EF- containing product (or for kittens could consider *Enterococcus hirae* instead if available) or oral capsule FMT if available. For *T. blagburni* infection, use specific EF containing synbiotic as an adjunctive to ronidazole. For young pedigree cats with protracted

diarrhoea with no discernible cause or a cryptic infection, consider Hill's Biome prescription food and/ or FMT (fresh enemas or capsules). For cats with constipation, options include SLAB51^{TM} probiotic blend and/ or psyllium as part of or alternative to a high fibre diet, or – based on recent evidence and subject to availability - potentially commercially available FMT capsules.

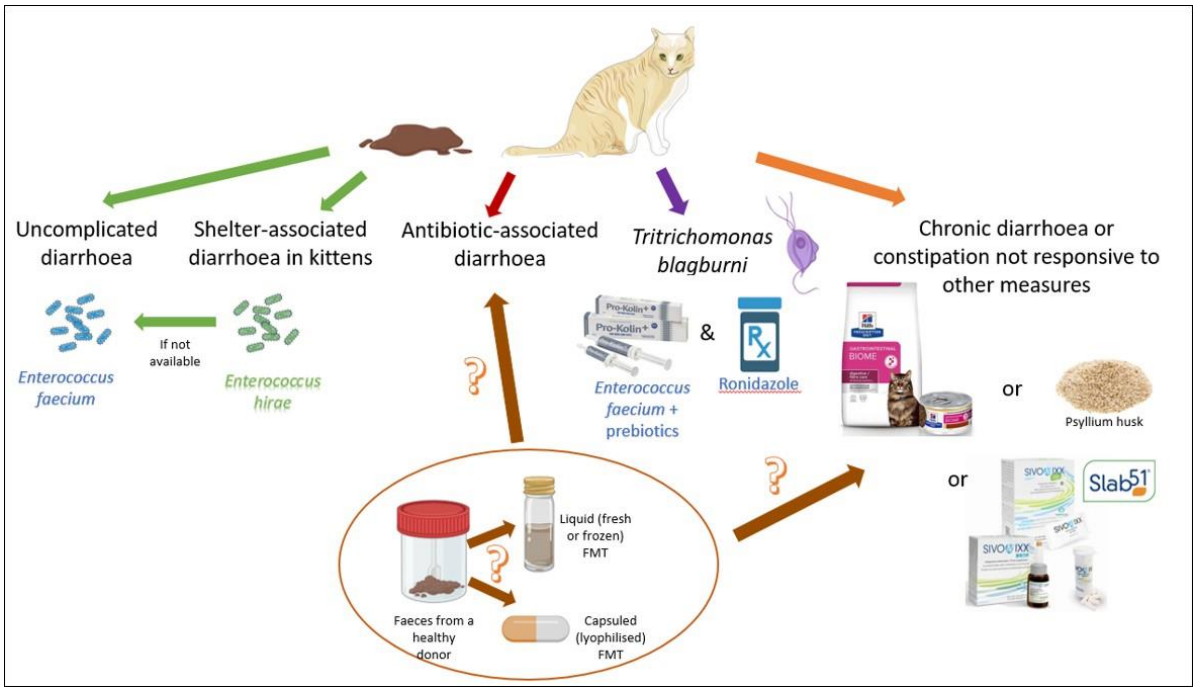

Figure 2. Proposed evidence-based use of specific pre- pro, and synbiotics as well as Faecal Microbiota Transplantation (FMT) in cats after appropriate diagnostic workup to rule out common aetiologies.

So where are we now? Overall, there is much more to learn about the feline microbiota and how to best manipulate it for both diagnostic and therapeutic purposes, but evidence for the use of pro- or synbiotics, dietary fibre/ prebiotics (in the form of additives or as part of available prescription diets), and possibly FMT in selected cases is slowly increasing. Prospective studies and standardisation of treatment, whether this is the type and dose of probiotics, or the type, dose and administration technique of FMT, are still needed to make more firm recommendations. There is an international consortium currently working on publishing these recommendations. Having said this, especially probiotics could be a safe and relatively risk-free alternative to common current treatments, especially in acute diarrhoea, and could reduce the unnecessary use of antimicrobials and associated risks (both to the individual but also to global health).

Key points summary/ take home messages:

- **There is limited evidence for the use of probiotics/ synbiotics in cats, but they can likely be used without side effects in the vast majority of uncomplicated cases with gastrointestinal signs**
- **The evidence-based use of dietary fibre supplements or high fibre diets is currently limited to cats with constipation**

- **There is only anecdotal evidence for the use of fresh faecal microbiota transplantation in specific conditions in cats, but it can be considered in situations where conventional treatment attempts have failed**
- **There is recent evidence that capsuled freeze-dried FMT can reduce clinical signs of diarrhoea, vomiting or constipation in a heterogeneous group of diseases in cats, but meaningful alterations of the intestinal microbiota were not achieved with this**

Case notes

Case description:

A 7 month old female spayed Ragdoll/ Bengal cross breed cat is presented for a 6-week long history of diarrhoea. She has been in the owner's possession since being a kitten, and there are no known similar concerns in any of the littermates. She is an indoor cat with lead-controlled access to the garden. There are no other animals in the household and she has never travelled outside the UK. She gets regularly treated for endo- and ectoparasites, the last course of fenbendazole was given 2 months ago. She is fed a prescription "gastrointestinal" diet and is having a good appetite. 6 weeks ago, the cat was looked after by someone else while owners were absent; when they returned the cat had soft to watery, yellow, sometimes slightly firmer and browner diarrhoea with no mucus or fresh blood, but occasional tenesmus and pain on defecation and a defecation frequency of 4-7 times/ day, and some episodes of accidents overnights where watery faeces were found near the litter tray. No other problems have been observed. The referring veterinarian has performed a faecal parasitology + Giardia antigen testing (negative), a faecal culture for salmonella and campylobacter (negative) and a faecal pathogen PCR panel, which was negative for Toxoplasma gondii, Tritrichomonas blagburni, Cryptosporidium, Giardia, Salmonella, FPV, and Cl. perfrigingens enterotoxin, but positive for Cl. perfringens alpha toxin and FCoV.

On physical examination, the cat is bright alert and responsive, with a heart rate of 180 bpm, respiratory rate of 36 bpm, a temperature of 36.2C, mucus membranes are pink and moist with a CRT < 2 sec, thoracic auscultation and abdominal palpation are unremarkable, the abdomen is soft and comfortable. Peripheral lymph nodes are of normal size. The cat has a BCS of 5/9 with a body weight of 3.48 kg.

How would you proceed?

Initial tests performed:

- Haematology and biochemistry including total T4: unremarkable
- FeLV/FIV in house Snap test: negative
- Toxoplasma gondii IgG/IGM: < 50 and < 20, respectively (= negative)
- Feline serum TLI: 34.8 ug/L (range 12.1-82 ug/L)
- Serum cobalamin: > 1000 ng/L (range 270-1000 ng/L)
- Abdominal ultrasound: Generalised marked hypoechogenicity of all lymph nodes with borderline size of medial ileac, jejunal and colic lymph nodes (0.40-0.49 cm). Perinodal

tissues are normoechoic. Prominent and mildly hypoechoic pancreas. Otherwise unremarkable
- Cytological examination of fine needle aspirates from jejunal lymph nodes: reactive lymphoid hyperplasia

The cat was discharged with the recommendation to switch to a commercial hydrolysed complete prescription food, but after 2 months strictly on this food, no improvement was noted. Another diet switch to "Biome" food was performed with no success, in fact, diarrhoea worsened and there was frequent faecal incontinence.

How would you proceed?

Further workup performed:

- Repeat faecal parasitology: negative
- Repeat abdominal ultrasound: mild thickening of ileal and colonic wall with preservation of normal layering, mildly progressive generalised lymphadenopathy (0. 6-0.9 cm)
- Repeat jejunal lymph node cytology: reactive hyperplasia
- Serum fPL: normal
- Colonic flush performed for direct microscopy (no protozoa visible) and *Tritrichomonas* PCR
- At the same time as colonic flush: 55 ml of fresh faecal microbiota transplantation slurry (donor faeces mixed 1:5 with saline and sieved) administered rectally
- *Tritrichomonas* PCR later received as: positive

Follow up:

The owner did not want to proceed with ronidazole treatment at this point, and the cat improved within a week after FMT administration (while remaining on Biome diet). Clinical signs (softer faeces, increased frequency, occasional haematochezia) alongside overgrooming recurred 3 months later. Probiotic SLAB51^{TM} was added and signs remained stable for another 3 months, when they started to become worse, with intermittent watery stools and more tenesmus and haematochezia. At this point it was decided to start ronidazole at 30 mg/kg once daily for 14 days. An *Enterococus faecium* containing synbiotic was given alongside and for 1 month after. Defecation frequency reduced to once daily with normal consistency and remained normal for 1 month, and then returned to 3-4 defecations a day with relatively normal consistency, which is stable to this day. Repeat *Tritrichomonas* PCR from faeces was negative.

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