



Review Article

In Vitro Models of the Canine Digestive Tract as an Alternative to In Vivo Assays: Advances and Current Challenges

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Abstract

Dogs occupy a full place in the family, and their well-being is of paramount importance to their owners. Digestion, a complex process involving physicochemical, mechanical, and microbial parameters, plays a central role in maintaining canine health. As *in vivo* studies in dogs are increasingly restricted by ethical, regulatory, societal, and cost pressures, an alternative option is the use of *in vitro* models simulating the different compartments of the canine gastrointestinal tract. This review introduces digestion and gut microbiota as key factors in dog nutrition and health under both healthy and diseased conditions (obesity and inflammatory bowel disease) and highlights similarities and differences between the human and canine digestive tract and processes. We provide the first in-depth description of currently available models of the canine digestive tract, discuss technical and scientific challenges that need to be addressed, and introduce potential applications of *in vitro* gut models in the food and veterinary fields. Even if the development of some *in vitro* models is still limited by a lack of *in vivo* data in dogs that is necessary for relevant configuration and validation, translation of long-term expertise on human *in vitro* gut models to dogs opens avenues for model optimization and adaptation to specific canine digestive conditions associated with various dog ages, sizes, breeds and/or diets, in both physiological and diseased states.

1 Introduction

1.1 Dogs in familial and economical contexts

Canis lupus familiaris, the domesticated dog, belongs to the *Canidae* family. The dog is thought to have been the first animal domesticated by humans around 12,000 years ago (Axelsson et al., 2013). Dogs were initially strict carnivores, but they probably acquired the ability to digest starch during the agricultural revolution to become facultative carnivores (Axelsson et al., 2013). Nowadays, canine species include approximately 400 breeds with broad morphological and size variabilities and body weight ranging from 1 kg for a Chihuahua to 100 kg for a Saint-Bernard (Grandjean and Haymann, 2010). Dogs occupy a full place in the family, and their health and well-being are of paramount importance to their owners.

There are estimated to be more than 500 million dogs worldwide, which represents a huge market for the petfood and animal health industry. In 2018, the global petfood market size reached

\$91.1 billion, representing a 31% increase within 5 years (Phillips-Donaldson, 2019). A vast array of foods, snacks, and nutritional supplements have been developed to support well-being, health, improve aging, or prevent disease. According to the increased interest of owners in maintaining their dogs' health, petfood has been adapted to fit each dog's lifestyle, for example specific products for puppies or lactating bitches, sedentary or active animals, and maintenance or hypocaloric diets.

Different types of canine food are available and can be classified into three categories: dry food, canned food, and alternative food (biologically appropriate raw food (BARF), homemade food, and feedstuffs). At the interface between petfood and veterinary compounds, nutritional supplements represent an expanding market with a huge product range. As an example, micronutrients like selenium, taurine or polyphenols can be added for old dogs, calcium, phosphorus, omega-3 fatty-acids and vitamin E for lactating bitches, or L-carnitine for athletic dogs. Supplements for specific nutri-

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tional purposes such as prebiotics or probiotics are also developed to decrease food sensitivities or for digestive or articular care.

In 2019, pet medicine, including vaccines, antiparasitic treatments or antibiotics, represented a \$17.5 billion market¹. Drugs can be administered in different forms: topically, by injection, or as oral formulations. Oral formulations can have liquid (e.g., solution or suspension), semi-solid (oily or aqueous formulations), or solid (e.g., powder, capsules, conventional or sustained-release tablets) forms. Specific treatments have been developed to avoid lipid absorption in obesity or decrease pain in inflammatory bowel disease (IBD). Of note, vulnerability to specific diseases depends on a dog's size and breed, with for instance obesity and dental trouble common in small dogs and digestive problems more common in larger animals.

1.2 Regulatory context

In Europe, the European petfood industry federation (FEDIAF) represents 16 national petfood industries and provides a framework for the production of safe, nutritious, and palatable petfood. Heads of medicine agencies or national agencies must evaluate the quality and safety of medicinal products for animals, consumers, users, and the environment as well as the effectiveness of the medicinal product before it can be marketed.

To assess the digestibility of petfood or bioaccessibility of active compounds (including drugs) in the canine gastrointestinal tract (GIT), *in vivo* studies remain the gold standard. *In vivo* experiments in dogs are also performed to model the human gut due to similarities in digestive physiology (Lui et al., 1986; Akimoto et al., 2000). More than 17,000 experiments were carried out on dogs in 2018 in the EU28 and Norway². However, *in vivo* assays are increasingly restricted by regulation, ethical and societal constraints, and high associated costs.

The 3Rs proposed by Russel and Burch (1959) widely encourage a reduction in the number of animals used in research and promote the development of alternative *in vitro* approaches. Among *in vitro* alternatives, models simulating the canine digestive environment (intestinal cell culture, organoids, or *in vitro* gut models) can help to answer many scientific questions associated with food and drug behavior during canine digestion, especially on mechanistic aspects. However, this approach requires a comprehensive understanding of the canine digestive process.

1.3 Digestion and gut microbiota as key parameters in dog nutrition and health

Canine digestion involves physicochemical (e.g., pH, digestive secretions, transit time), mechanical, and microbial parameters. These digestive components affect food digestibility, nutrient absorption, and energy release, but also drug metabolism and absorption, and survival of probiotic microorganisms. Thus, the development of new petfood or veterinary products needs to con-

sider these multi-faceted aspects of canine digestion to answer important questions such as: How do physicochemical parameters modulate food digestibility? What is the importance of gut microbiota in canine digestion and drug metabolism? Where are drugs released and absorbed? How is drug bioaccessibility impacted by food matrix, galenic form, physicochemical parameters, or microbiota? How do probiotic strains survive along the GIT? However, recommendations for petfood and drug intake are currently based only on dog body weight or metabolic weight. Development of new products should consider the variations in the digestion process associated with different canine sizes and breeds to move towards personalized nutrition and veterinary medicine.

2 Methods of literature research and aim of the review

Our literature search was performed using *PubMed*³ and *Google Scholar*⁴ using the keywords “dog” OR “canine” AND “digestion”, “pH”, “enzyme”, “digestive secretions”, “absorption”, “microbiota”, “bile acids”, “transit time”, “short chain fatty acids”, “fermentation”, “gas”, “mucus”, “*in vitro*”, “model” in all available years. The online database search was last performed in August 2021 on titles, abstracts, and key words including original articles, reviews, theses, and books. Relevant studies were identified after consultation of the main text, figures and supplementary materials, and information regarding involved dogs (i.e., number of dogs, age, weight, breed, sex, reproduction state, living environment), alimentation (i.e., type of food, feeding frequency, principal components of food), health (i.e., healthy, obese or IBD dogs only), and analysis methods was extracted.

This review paper aims to give a state-of-the-art on canine digestive physiology regarding both physicochemical and microbial parameters that can be reproduced in *in vitro* gut models. Then, we explain how these parameters change under diseased conditions associated with obesity and IBD. In a third part, we provide an in-depth description of all available *in vitro* models of the canine digestive tract before discussing their limitations and challenges associated with the development of *in vitro* gut models of healthy or diseased dogs and their applications in the food and veterinary fields. This paper considers the entire canine GIT and associated microbiota and highlights similarities and differences between dog and human digestive physiology to provide new opportunities for canine *in vitro* gut simulation.

3 Canine digestion

3.1 Digestive anatomy

Because dogs are facultative carnivores, their GIT is adapted to high-protein and high-fat diets, i.e., relatively short and simple

¹ Animal medicine global market opportunities and strategies to 2023. <https://www.globenewswire.com/news-release/2020/01/13/1969734/0/en/Global-Animal-Medicine-Market-Opportunities-Strategies-to-2023-Veterinary-Pharmaceuticals-Will-Gain-20-7B-of-Global-Annual-Sales-by-2023-A-6-6B-Opportunity-for-Veterinary-Parasitic.html>

² https://webgate.ec.europa.eu/envdataportal/content/alures/section1_number-of-animals.html (accessed 03.02.2022)

³ <https://pubmed.ncbi.nlm.nih.gov>

⁴ <https://scholar.google.fr>

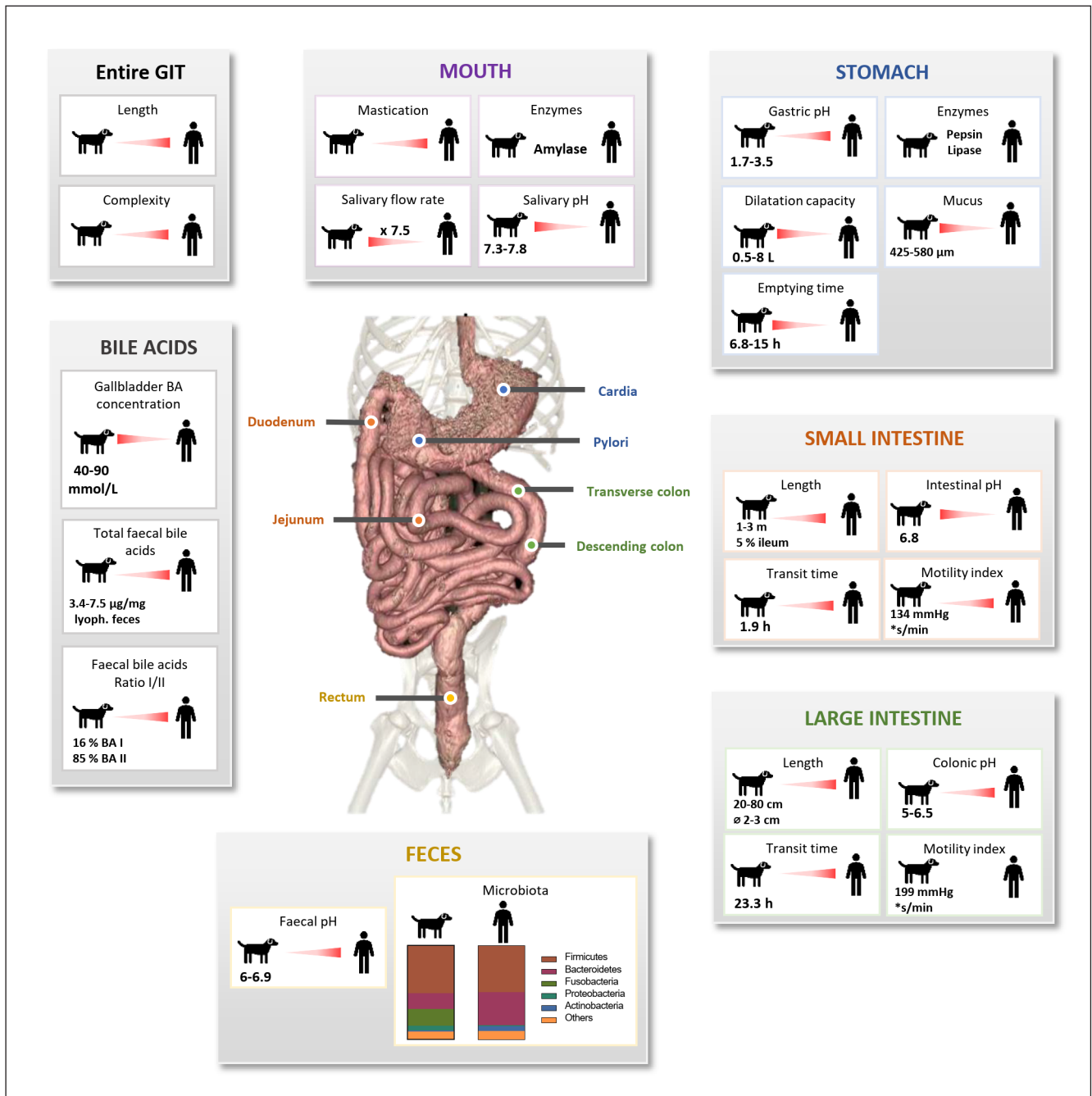


Fig. 1: Canine digestive compartments and associated mechanical, physicochemical, and microbial processes

Key parameters of the oral, gastric, intestinal, and colonic compartments of medium-sized healthy dogs are summarized and compared to *in vivo* data in healthy humans. Lack of *in vivo* data is represented by “?”. BA, bile acid

compared to herbivores and even omnivores like humans or pigs, reflecting the lower retention time required for meat in comparison to grass digestion (Moon et al., 2018) (Fig. 1). A dog’s digestive tract weight in relation to total body mass is inversely correlated to canine size, representing 7% and 2.8% of the total body weight in 5 and 60 kg dogs, respectively (Weber, 2006). The GIT

length is correlated with shoulder height at a 6:1 ratio (Morris and Rogers, 1989).

Digestion in dogs starts in the mouth with the mechanic action of mastication, using 42 teeth and only 2000 taste buds. The canines can cut meat into pieces. The molars have a larger crown than in humans and allow grinding of bones. The mastication step



is less important than in humans as most dogs do not really chew but swallow large pieces of food. After swallowing, the food bolus crosses the esophagus, which has a length similar to that of the human esophagus, i.e., around 30 cm for a medium-sized dog (Kararli, 1995; Freiche and Hernandez, 2010). Like in humans, the canine stomach has the shape of a J-elongated bag. It is located in the abdomen, starts with the cardia and extends to the pylorus, and is composed of the antrum and the fundus (Kararli, 1995). Its dilation capacity (0.5-8 L volume) is larger than that of the human stomach, and the gastric wall measures between 3 and 5 mm in thickness (Kararli, 1995; Freiche and Hernandez, 2010).

Digestion continues along the small intestine, which is anatomically divided into the duodenum (10%), jejunum (85%) and ileum (5%), measuring from 1 to 3 m total length. In comparison, the human ileum represents 60% of the total small intestine length (Kararli, 1995; Oswald et al., 2015). This suggests that ileal function may differ between dogs and humans. Canine small intestine diameter is smaller than in humans (1 cm versus 5 cm). Duodenal thickness reaches 6 mm, whereas intestinal loops measure around 2-3 mm. The large intestine or colon is 20-80 cm in length with a diameter of 2-3 cm in medium-sized dogs, while it is 90-150 cm in humans with a diameter of 5 cm (Kararli, 1995). The large intestine is composed of the ascending, transverse, and descending colon. Finally, the rectum forms the terminal section of the large intestine ending in the anus. The three parts of the canine colon are less well defined than in humans, with the particularity of being non-sacculated and devoid of a sigmoid colon (Kararli, 1995).

As for humans, peripheral organs are involved in canine digestion. The pancreas secretes pancreatic juice into the duodenum and is involved in protein (trypsin, chymotrypsin, elastase, and carboxypeptidase), carbohydrate (α -amylase), and lipid (lipase and phospholipase) digestion. Pancreatic juice also contains antimicrobial agents that contribute to microbial balance. The liver is coupled with a gallbladder and located near the stomach. The liver plays a central role in digestion, including vitamin and glucose storage (glycogen), detoxification and excretion of toxic substances (e.g., urea), and lipid digestion by saponification of lipids by bile acids (BA). Bile is produced by the liver, stored in the gallbladder, and discharged into the duodenum.

Depending on body size and breed, dogs show a diversity in anatomical features such as intestinal length and volume, intestinal villus morphology, and intestinal or colonic permeability (Zentek and Meyer, 1995; Oswald et al., 2015). Because of the relationship between anatomy and digestive physicochemical parameters, these differences may affect dog digestion and key parameters such as pH, digestive secretions, transit time, and gut microbiota. To avoid making this discussion too complex, the following sections (3.2 and 3.3) focus on digestion processes in medium-sized dogs (from 10 to 30 kg).

3.2 Physicochemical parameters

pH

Gastrointestinal pH changes along the dog's digestive tract (Fig. 1). The mean salivary pH of medium-sized dogs is 7.3-7.8 (Smeets-Peeters et al., 1998). In the stomach, the arrival of a food bolus induces the secretion of gastrin, which in turn stimulates

hydrochloric acid (HCl) production. The gastric pH of Beagles under fasted conditions is around 1.5 (range 0.9-2.5), similar to that of humans (range 1.4-2.1) (Dressman, 1986; Kararli, 1995; Mahar et al., 2012). Several studies have observed a higher gastric pH in fed dogs, ranging from 2 to 5.5 (Smith, 1965; Dressman, 1986; Kararli, 1995; Shinchi et al., 1996; Martinez, 2002; Duysburgh et al., 2020). In the small intestine, the pH increases to values close to neutrality because of the buffering capacity of pancreatic juice and bile (Kararli, 1995). The pH of the small intestine also increases from the proximal to the distal part, from 6.5 to 8 in medium-sized dogs (Koziolek et al., 2019). The few studies that have investigated the canine jejunal pH measured a mean pH of 6.8 for medium-sized dogs (Mentula et al., 2005; Kalantzi et al., 2006), which is similar to that of humans (value of 6-7) (Kararli, 1995; Martinez, 2002). Colonic pH is, like in humans, more acidic with values of 5-6.5 (Smith, 1965; Koziolek et al., 2019). The fecal pH of medium dogs has a pH range of 6-6.9 (Eisenhauer et al., 2019; Nogueira et al., 2019).

Digestive secretions

Dog digestion is accelerated by digestive secretions containing various enzymes (Fig. 1). Amylase, lactate dehydrogenase and adenosine deaminase are found in dog saliva (Lavy et al., 2012; Iacopetti et al., 2017; Ricci et al., 2018). The gastric mucosa secretes gastric juice containing proteolytic (pepsin, chymosin) and lipolytic (lipase) enzymes (Aspinall, 2004; Durand, 2010). Canine pancreatic juice contains amylase, lipase, phospholipases, cholesterases, proteases, and nucleases (Kienzle, 1988; Robin, 2007). Of note, gastric and pancreatic juices are poorly characterized in dogs, in contrast to bile, for which more data are available. Bile is discharged into the duodenum during postprandial phases. Bile is up to 10-fold more concentrated in the gallbladder than in the liver, with a total concentration around 50 (40-90) mmol/L in dogs versus 3-45 mmol/L in humans (Nakayama, 1969; Kararli, 1995; Kakimoto et al., 2017; Nagahara et al., 2018; Larcheveque, 2019). Canine bile contains up to 15 BA, but the three major BAs (taurocholic, taurodeoxycholic and taurochenodeoxycholic acid) found in healthy dogs make up 99% of the total BA pool (Washizu et al., 1994). 99.04% of BA are conjugated to taurine, 0.15% to glycine and the remaining 0.81% are unconjugated (Nagahara et al., 2018). Fecal samples show concentrations of 5.8-7.5 μ g total BA per mg of dry feces (Schmidt et al., 2018; Blake et al., 2019; Manchester et al., 2019). Lastly, as in various mammals including humans, mucins are produced by goblet cells all along the canine GIT (Kararli, 1995). Mucus thickness has been evaluated in dogs only in the stomach, measuring 425 and 576 μ m respectively in the fundus and antrum in agreement with human values (Bickel and Kauffman, 1981; Kararli, 1995; Etienne-Mesmin et al., 2019). The mucin layer protects the epithelium from the acidic pH of the stomach and withstands bone fragments (Moon et al., 2018). In the large intestine, the mucus layer is colonized by a microbial biomass, as described in Section 3.3.

Gut motility and transit time

Using wireless motility capsules, Farmer and collaborators found that gut motility indexes in dogs are higher in the large

intestine (199 mmHg*sec/min) compared to the small intestine (134 mmHg*sec/min) and stomach (55 mmHg*sec/min) (Farmer et al., 2018). Canine motility is quite different to that of humans. Maximum pressure under fed conditions is higher in humans than in dogs (241 and 119 mmHg in the human stomach and small intestine, respectively, versus 52 and 75 mmHg for dog), but with a similar maximum frequency of 3.7 contractions/min in the gastric compartment (Boscan et al., 2013; Farmer et al., 2018). Data on transit time in the different digestive compartments of dogs (Fig. 1) vary widely depending on the method used (e.g., radiopaque markers, plastic beads, ^{13}C -octanic acid breath test, sulfasalazine-sulfapyridine method, or wireless motility capsule), breed, age, feed (composition, energy density or viscosity) and environment (laboratory or owners' home, stress context or sedation). Gastric emptying time varies from 2-19.8 h in fed medium-sized dogs (Hinder and Kelly, 1977; Theodorakis, 1980; Ehrlein and Pröve, 1982; Meyer et al., 1985; Lui et al., 1986; Gupta and Robinson, 1988; Hornof et al., 1989; Arnbjerg, 1992; Carrière et al., 1993; Cullen and Kelly, 1996; Sagawa et al., 2009; Boillat et al., 2010; Mahar et al., 2012; Boscan et al., 2013; Koziolek et al., 2019). Small intestinal transit time has been reported to have a range of 1.1-3.6 h (Boillat et al., 2010; Oswald et al., 2015). Large intestine transit time in dogs ranged from 1.1-49.1 h measured in dogs from a wide range of weights and breeds (Boillat et al., 2010; Warrit et al., 2017).

3.3 Canine gut microbiota

Composition

Microorganisms colonize the entire GIT of dogs from mouth to rectum. Like in humans, there are longitudinal variations (i.e., along the GIT) in gut microbiota composition due to changes in pH, substrate concentrations (including oxygen and nutrient availability), and transit time (Hooda et al., 2012; Friedman et al., 2018; Etienne-Mesmin et al., 2019). Figure 2 gives an overview of available data on gut bacteria composition of the different gastrointestinal regions in dogs.

The stomach is the least colonized compartment with 10^4 to 10^5 colony forming units (CFU) per gram of content in medium-sized dogs, mainly composed by *Proteobacteria*, including *Helicobacter* spp. as in humans, which are potential pathogenic strains (Benno et al., 1992; Mentula et al., 2005; Hooda et al., 2012).

The small intestine contains 10^5 to 10^7 CFU/g of content (Benno et al., 1992; Mentula et al., 2005). The duodenum is colonized by *Firmicutes* (calculated median of the three available publications: 47%), *Proteobacteria* (27%), *Bacteroidetes* (9%), *Fusobacteria* (3%), and *Actinobacteria* (1%), whereas the jejunum is characterized by a higher abundance of *Proteobacteria* (37%), *Actinobacteria* (11%), and *Fusobacteria* (10%), together with lower percentages of *Firmicutes* (33%) and *Bacteroidetes* (7%) (Xenoulis et al., 2008; Suchodolski et al., 2009; Garcia-Mazcorro et al., 2012). A single study on 6 dogs investigated ileal microbiota and observed 31% *Fusobacteria*, 24% *Firmicutes*, 23% *Bacteroidetes* and 22% *Proteobacteria* (Suchodolski et al., 2008).

The colon is the most colonized part of the GIT, with up to 10^9 - 10^{11} CFU/g of content (Hooda et al., 2012). According to a unique publication using 16S Illumina sequencing to investigate microbi-

ota composition, colonic digesta is dominated by 37% *Firmicutes*, 33% *Bacteroidetes*, 29% *Fusobacteria* and 1% *Proteobacteria* (Suchodolski et al., 2008a).

The majority of taxa colonizing the colon are also found in canine feces (Pilla and Suchodolski, 2020), which seems to be rather different from the human situation, where a significant number of mucus-adherent bacteria from the colon are not found in feces (Pilla and Suchodolski, 2020). In addition to bacteria (98%), canine fecal microbiota contains 1.1% archaea, 0.4% fungi and 0.4% viruses, mainly bacteriophages (Suchodolski, 2011; Swanson et al., 2011). Fecal microbiota of healthy dogs is dominated by three main bacterial phyla: *Firmicutes*, *Bacteroidetes* and *Fusobacteria* (Pilla and Suchodolski, 2020). Bacteria from *Actinobacteria* and *Proteobacteria* phyla are also found in canine feces but in a lower proportion.

Fusobacteria and *Proteobacteria* seem to be more abundant in dogs than in humans, probably related to a carnivorous versus omnivorous diet (Fig. 1) (Simon, 2019). Unlike in humans, where *Fusobacterium* is frequently associated with diseases, in dogs this genus is related to non-stressful conditions and therefore probably a healthy state, especially because its abundance increases when dogs have access to the outdoors (Oswald et al., 2015).

In addition to longitudinal variations, scarce data suggest variations in microbiota composition from the digestive lumen to the surface of the intestinal epithelium covered by a mucin layer. Only two studies have investigated the mucosa-associated bacteria on the outer mucus layer in the colon of healthy dogs (Simpson et al., 2006; Cassmann et al., 2016). Cassmann et al. (2016) demonstrated that free colonic mucus is mainly colonized by *Bacteroidetes* spp. and *Eubacteria*. Of interest, *Akkermansia muciniphila*, a well-known mucin-degrading bacterium in humans, which is inversely correlated with obesity, was not yet identified in canine feces (Garcia-Mazcorro et al., 2020).

Metabolic activities

Gut microbiota is known to play a key role in host homeostasis and health maintenance, as it is implicated in many nutritional (e.g., fiber degradation and vitamin synthesis), immunological (immune system maturation) and physiological processes (e.g., vascularization, epithelium integrity, barrier effect against pathogens, and lipid digestion via the metabolism of primary BA into secondary BA) (Durand, 2010; Andoh, 2016). Gut microbiota metabolic activity leads to short-chain fatty acid (SCFA) and gas production as mean fermentation products resulting from degradation of soluble fibers. In dogs, like in humans, the three main SCFAs are acetate, propionate and butyrate, with relative percentages in fecal samples of 60:25:15 (Mondo et al., 2019). Non-digested proteins from diet and endogenous proteins (e.g., from mucins) are also metabolized by gut microbiota, leading to the production of branched chain fatty acids (BCFA), ammonia, indoles and phenols (Weber et al., 2017). Total fecal SCFA and BCFA were investigated, with values widely varying between studies, i.e., 91-423 and 4.7-36.1 $\mu\text{mol/g}$ of lyophilized stools, respectively (Beloshapka et al., 2012; Minamoto et al., 2015; Alexander et al., 2019; Detweiler et al., 2019; Eisenhauer et al., 2019; Nogueira et al., 2019). To our knowledge, there is no *in*

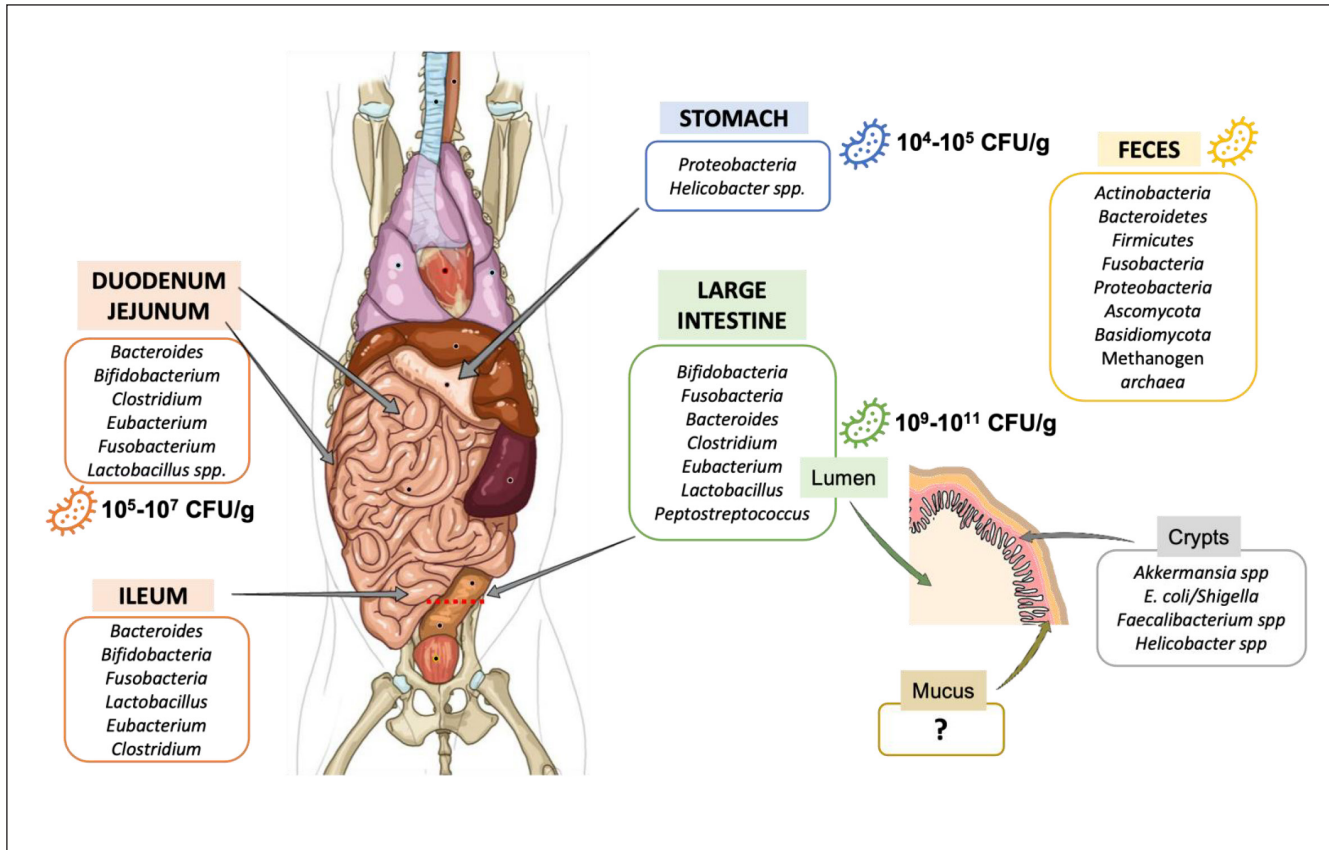


Fig. 2: Variations in gut microbiota composition along the canine digestive tract

Main bacterial populations found in the different compartments of the gastrointestinal tract of the medium-sized dog are represented. CFU, colony-forming unit

in vivo data on gas production in dogs, and the two available studies on gas composition focused on malodorous compounds such as hydrogen sulfide (Collins et al., 2001; Giffard et al., 2001).

4 From healthy to diseased dogs: impact on digestive physiology and gut microbiota?

As in humans, diseases such as obesity and IBD are increasingly prevalent in dogs. Because the etiology of such diseases is now better understood in humans, the emergence of similar pathologies in dogs has raised awareness of veterinary and petfood companies and motivated development of pharma and nutritional products. Most available data on obese or IBD dogs focus on the lower gut and its associated microbiota. Indeed, in many intestinal or extra-digestive diseases such as obesity and IBD, in dogs like in humans, gut microbiota appears to be in a disrupted state called “dysbiosis” where both functional and compositional states are altered (Pilla and Suchodolski, 2020).

4.1 Obesity

In Western countries, obesity is considered the most common nutritional disorder in pets due to an imbalance between energy in-

take and expenditure (Osto and Lutz, 2015). Dogs are considered clinically obese when their body weight is at least 20-30% above ideal weight, and a universal body condition score (ranging from 1 to 9) defines overweight dogs at 7 and obese dogs at 8/9 (Apper et al., 2020). Both sexes have a similar incidence and all dogs are affected whatever their size, even if certain breeds seem to be more predisposed, like labrador, retriever, Bernese mountain dog, Cavalier King Charles or beagle (Osto and Lutz, 2015). Canine obesity is generally associated with insulin resistance, altered lipid profile, hypertension, orthopedic and cardiorespiratory disease, and development of low-grade systemic inflammation (Tvarijonaviciute et al., 2012). While gene mutations are associated with increased body weight in beagle and labrador, diet is clearly a determinant of obesity through excess energy intake (Zeng et al., 2014; Raffan et al., 2016).

Several mechanisms may implicate gut microbiome in the onset and evolution of dog obesity. Based on human and murine data, this includes higher energy utilization from non-digestible carbohydrates, manipulation of host gene functions, and exacerbation of inflammation (Hamper, 2016). Five studies have compared fecal microbiota composition of obese and lean dogs (Fig. 3). Using 16S rRNA gene pyrosequencing in companion and laboratory dogs, dominance of *Firmicutes* (> 90%) was observed

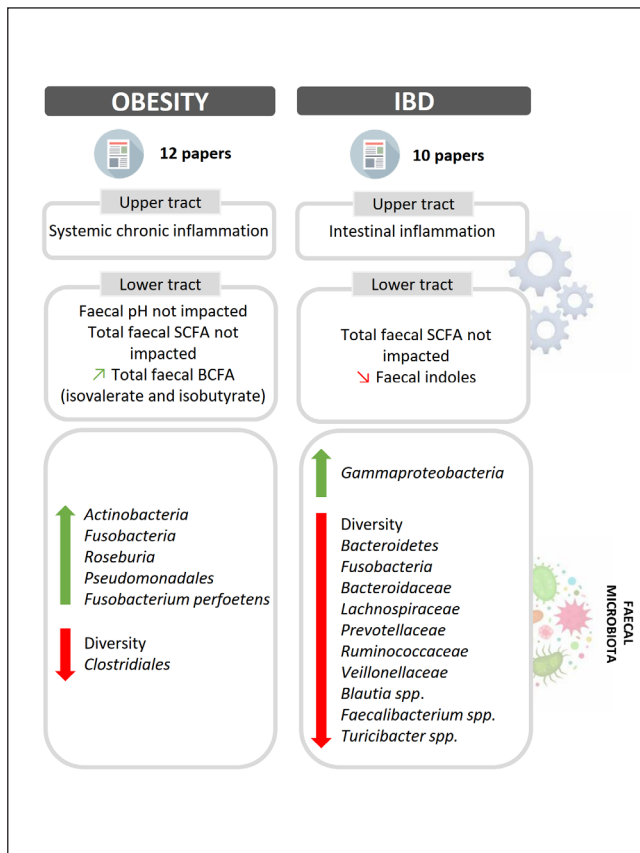


Fig. 3: Impact of obesity and inflammatory bowel disease on canine digestive physiology

Main variations in upper and lower digestive physiology and changes in fecal microbiota composition associated with canine obesity and IBD are represented. Increased parameters are indicated by green arrows, decreased parameters by red arrows. BCFA, branched-chain fatty acids; IBD, inflammatory bowel disease; SCFA, short-chain fatty acids

in both obese and lean groups, but mean abundance of *Actinobacteria* and *Roseburia* was greater in obese dogs (Handl et al., 2013). Using the same method, Salas-Mani et al. (2018) showed that *Proteobacteria* was predominant in obese dogs (76%), whereas fecal microbiota from lean dogs was mainly composed of *Firmicutes* (85%). In addition, *Clostridiales* appeared to be less abundant in obese compared to lean dogs, while the opposite result was found for *Pseudomonadales*. However, in a recent study involving 17 healthy and 22 obese companion dogs, no significant difference in any taxa was highlighted when comparing the two groups using Illumina 16S rRNA gene sequencing (Forster et al., 2018). With the same method, Bermudez Sanchez and colleagues (2020) described a relative abundance of 92% of *Firmicutes*/Bacteroidetes ratio of 0.123 in 20 obese dogs (Bermudez Sanchez et al., 2020). Lastly, in a very recent study, a positive association between *Fusobacteria* level, especially *Fusobacterium perfoetens*, and body score condition in 24 overweight

laboratory beagles was established using metagenomic analysis (Chun et al., 2020). In dogs, like in humans, microbial diversity seems to decrease in obese compared to lean dogs, with a Shannon index of 1.3 and 2.3, respectively (Park et al., 2015). However, more investigations are required to characterize canine obese microbiota, in feces but also in other digestive compartments, to determine if some species should be used as obesity markers. As an example, *Proteobacteria* were recently correlated with increased weight in overweight dogs, as has been suggested in humans (Apper et al., 2020).

In addition to microbiota changes, other physiological modifications have been demonstrated in obese humans compared to healthy individuals, including an increase in BA production by liver (coupled with high amounts of primary BA in stool), and a higher SCFA production, leading to a decrease in colonic pH (Rahat-Rozenbloom et al., 2014). Due to similarities between dog and human GIT and lifestyle, it would be interesting to establish whether similar phenomena occur in obese dogs. Mean fecal pH of obese companion beagles (6.6) was not significantly different from that of lean dogs (6.8) whatever the diet, i.e., high-fat or low-fat diet (Xu et al., 2017). In the same study, total fecal SCFA concentration was also equivalent in the two groups, whereas fecal BCFA such as isovalerate and isobutyrate were significantly more concentrated in obese compared to lean dogs. To our knowledge, no study has investigated BA production in obese versus lean dogs. However, recent studies recommend to monitor fecal BA concentrations along with microbiota in lean and obese dogs as they appear to be interesting markers of glucose homeostasis failure in obese dogs (Forster et al., 2018; Apper et al., 2020).

4.2 Inflammatory bowel disease

In dogs, IBD is classified as a chronic enteropathy, but defining its prevalence remains difficult because diagnosis of the disease is challenging. IBD is characterized by an irregular alternation of clinically active (with pain and diarrhea) and insignificant phases. Outside active phases, there are recurring gastric symptoms with histopathological changes in mucosa of the small and large intestines (Malewska et al., 2011). The predominant causes of canine IBD include bacterial and environmental factors, genetic predisposition of selected breeds, food allergies, and some drugs (Malewska et al., 2011). Pathogenesis of IBD includes loss of tolerance for endogenous microbiota, chronic inflammation of the GIT associated with an increase in intestinal permeability, and immune cell infiltration in the *lamina propria* (Junginger et al., 2014). Clinical scenarios include a decline in activity level and appetite, vomiting, increase in stool frequency, loss of stool consistency (increase in fecal water content), and weight loss.

Recent reviews on IBD in dogs (Fig. 3) report modifications in gut microbiota structure (compared to healthy animals) similar to those found in humans (Xenoulis et al., 2008; Hooda et al., 2012; Suchodolski et al., 2008, 2010, 2012a,b; Cassmann et al., 2016; Pilla and Suchodolski, 2020), although Xu et al. (2016) did not observe significant differences between healthy and IBD groups. Minamoto and collaborators (2015) showed an overall decrease in fecal diversity in IBD compared to healthy dogs, as also de-



scribed in humans (Minamoto et al., 2015). Furthermore, a dysbiosis index designed using qPCR targeting of eight bacterial groups to assess fecal microbial changes associated with canine chronic enteropathies allowed discrimination between healthy and diseased dogs with 95% confidence range (AlShawaqfeh et al., 2017). Fungal DNA was more frequently detected in dogs with chronic enteropathies than in healthy animals. FISH analysis of colonic biopsies from boxers with granulomatous colitis revealed mucosa colonization by an unknown adherent and invasive *E. coli* strain (Simpson et al., 2006). The data indicate that global microbial structure and diversity, more than a single taxa, should be followed to discriminate healthy and IBD dog microbiota (Scarsella et al., 2020).

Concerning functional activity, there is no significant difference in fecal SCFA concentrations between IBD and healthy dogs, but a lower indole concentration was measured in diseased dogs (Xu et al., 2016; Pilla and Suchodolski, 2020). This is an important point because indoles have well-known anti-inflammatory effects, strengthen epithelial barrier, and decrease *E. coli* attachment to the epithelial wall (Chèvreton, 2018). Alterations in microbial functions associated with IBD were estimated using a prediction tool (PICRUSt) from 16S rRNA gene data, highlighting a significant increase in secretion system pathways and transcription factors (Minamoto et al., 2015). Other parameters modified in humans during IBD (Duboc et al., 2013; Rana et al., 2013; Fitzpatrick and Jenabzadeh, 2020) such as transit time and BA dysmetabolism (increase in fecal primary BA) have not been investigated in dogs yet.

5 *In vitro* canine models as an alternative to *in vivo* assays in dogs

5.1 Generalities on *in vitro* gut models: static versus dynamic and comparison with *in vivo*

A wide range of *in vitro* gut models has been developed, from simple static mono-compartmental models to more complex dynamic and multi-compartmental models (Guerra et al., 2012; Payne et al., 2012). These *in vitro* models have been primarily developed to mimic human digestion but have also been adapted to simulate animal digestion, mainly that of pig or piglet (Meunier et al., 2008; Tanner et al., 2014; Fleury et al., 2017; Dufourmy et al., 2019), cat (Sunvold et al., 1995a,b; Van den Abbeele et al., 2020a) or dog (Sunvold et al., 1995a,b; Smeets-Peeters et al., 1999; Tzortzis et al., 2004; Hervera et al., 2007; Bosch et al., 2008; Cutrignelli et al., 2009; Panasevich et al., 2013; Lee et al., 2017; Vierbaum et al., 2019; Oba et al., 2020; Van den Abbeele et al., 2020a; Verstrepen et al., 2021), as described in Section 5.2.

Simple static models of the upper gut (Minekus et al., 2014) reproduce the successive oral, gastric and/or small intestinal phases of human digestion in a single vessel maintained at body temperature by changing pH conditions and adding appropriate digestive secretions (e.g., α -amylase in the oral phase, pepsin and/or lipase in the stomach, and bile and/or pancreatic juice in the intestinal phase). The simplest models of the colon compartment are thermostatic batch culture systems. These models are

inoculated with feces to simulate colonic fermentation and are maintained under anaerobic conditions by flushing them with nitrogen or carbon dioxide without renewal of nutritive growth medium. Such approaches are therefore limited in the time they can be run by substrate availability (24 to 72 h), and parameters like pH or redox potential are not regulated.

Compared to static systems, dynamic models reproduce changes in at least one parameter such as pH kinetics, variation in digestive secretions, or chyme transit. They can be mono-compartmental or are composed of sequential vessels simulating the successive digestive compartments. Dynamic mono-compartmental models of the upper gut are only gastric digesters (Kong and Singh, 2010; Thuenemann et al., 2015), while multi-compartmental models include gastric and small intestinal compartments, most frequently simulating the duodenal section (Tompkins et al., 2011; Ménard et al., 2015). All these models only reproduce physicochemical parameters of the upper digestive tract, such as temperature, gastric and intestinal pH, gastric and ileal deliveries, transit time, digestive secretions and passive absorption of nutrients and water. Dynamic large intestine models are based on the principle of continuous or semi-continuous fermentation and are inoculated with fecal samples. Such models are maintained under anaerobiosis and reproduce colonic temperature, pH, and transit time, and redox potential can be monitored. Moreover, a nutritive medium aiming to mimic ileal effluents and composed of various complex sources of carbon and nitrogen, electrolytes, BA, and vitamins is continually added to the bioreactor while fermentation medium is regularly removed. This allows maintaining functional microbiota for up to several weeks (or even several months with specific adaptations) (Fehlbaum et al., 2015). Several configurations of these colon models include the use of three-stage bioreactors in series to mimic the different sections of the human colon (Gibson et al., 1988; Cinquin et al., 2006; Van de Wiele et al., 2015) or the addition of mucin beads to distinguish luminal from mucosal colonic environments and their associated microbiota (Van den Abbeele et al., 2009; Deschamps et al., 2020). Up to now, the TNO Gastro-Intestinal Model (TIM-1) is probably the most complete *in vitro* system, with its four compartments reproducing the stomach and small intestine of monogastrics (Minekus et al., 1995; Meunier et al., 2008; Denis et al., 2016), while only two models simulate the whole digestive tract from the stomach to colon: the Simulator of Human Intestinal Microbial Ecosystem (SHIME) (Molly et al., 1993; Roussel et al., 2020), and the SIMulator of the Gastro-Intestinal tract (SIMGI) (Barroso et al., 2015).

Despite the obvious limitations of *in vitro* approaches, i.e., no input from nervous, endocrine or immune systems, artificial gut models have many advantages in terms of low cost, technical flexibility, and reproducibility. Especially, the spatial compartmentalization of bi- and multi-compartmental models allows sample collection over time and in the desired segment of the GIT, while *in vivo* studies mainly provide endpoint measurements (e.g., in fecal samples), since access to the different segments of the digestive tract (from the stomach to proximal colon) is restricted. Besides, in canine *in vivo* assays, there is frequently a huge discrepancy between studies due to different diets, life-

style, sizes and breeds. Therefore, as in humans, inter-individual variability is one of the main challenges. In comparison, *in vitro* approaches enable a high level of experimental control and reproducibility by excluding confounding environmental or dietary factors and therefore allow in-depth mechanistic studies on pharma and food compounds.

5.2 Currently available canine *in vitro* gut models

Since 1995, twelve *in vitro* models of the canine gut have been developed (Tab. 1). There is no available model of the oral phase, and only three models mimic the upper GIT (Smeets-Peeters et al., 1999; Hervera et al., 2007; Lee et al., 2017).

In vitro models of the upper gut

While Hervera and colleagues (2007) simulate stomach and small intestine digestion in batch vessels by adding crushed dog food to pepsin and pancreatin secretions only, a very complete model of the canine upper gut was developed in 2000 based on the TIM-1 technology, initially set up to reproduce human digestive conditions. Though the TIM-1 model is the most complete simulator of the upper gut, it only reproduces physicochemical and not microbial digestive parameters. Another main limitation of this model is that tested food should be finely mixed before digestion, which can influence nutrient digestibility.

FIDO (for Functional gastroIntestinal DOg model) integrates all the upper digestive compartments (stomach, duodenum, jejunum and ileum) and simulates body temperature, kinetics of gastric and small intestine pH, half-time delivery of gastric and ileal compartment, transit time and chyme mixing, sequential delivery of digestive secretions (gastric juice containing *Rhizopus* lipase and porcine pepsin, porcine pancreatic juice, bovine trypsin, electrolytes, and porcine bile), and intestinal passive absorption through hollow dialysis fibers (Smeets-Peeters, 2000). FIDO was set-up to mimic canine digestive parameters of medium dogs based on literature review (Smeets-Peeters et al., 1998). All parameters were therefore adapted to *in vivo* data, except for temperature, which was kept at 37°C like in humans, and parameters of passive absorption, probably due to a lack of data. The model was validated only for nutritional applications, by comparing protein digestibility and calcium bioaccessibility in the FIDO model and in ileal cannulated dogs (5 dogs).

More recently, the Artificial Stomach-Duodenum (ASD) dissolution model was adapted to dog digestion for formulation solubility studies (Lee et al., 2017). This bi-compartmental model, set at 37°C, simulates both the stomach and duodenum with associated pH (6.8 and 6.8-7, respectively), transit time (adapted from *in vivo* data), and pancreatic secretions. In this model, the gastric pH is particularly high compared to the other two (6.8 for the ASD model versus pH 2 for Hervera's model and 2-6 for FIDO).

In vitro models of the lower gut: batch and continuous models

Eight other available devices are *in vitro* static models of the canine colon based on batch fermentation. The simplest model of the colon of adult dogs was developed by Cutrignelli et al. (2009). Here, a single vessel was inoculated with diluted feces from adult large dogs and maintained at 39°C under anaerobiosis

without any addition of nutritional medium except for the tested carbohydrates. Another batch model set up by Sunvold et al. (1995c) to reproduce the colon of adult medium-sized dogs used a simulated growth medium adapted to dog digestion. This model was validated against *in vivo* data from 30 medium-sized dogs regarding fiber digestibility and production of SCFAs. Another static model, a batch system inoculated with dog fecal samples (no information on age and size of animals), was recently developed by Van den Abbeele et al. (2020a). A nutritive medium was introduced to simulate dog ileal effluents, but this was similar to one used previously for human experiments (Van den Abbeele et al., 2018). Batch models developed by other teams share the limitation that they lack a nutritive medium adapted to the canine situation (Tzortzis et al., 2004; Panasevich et al., 2013; Vierbaum et al., 2019). Bosch et al. (2008) developed a more complete batch fermentation model mimicking the ileum, proximal colon, transverse colon, or rectum compartment in different vessels inoculated with corresponding digestive fluids from 3 adult small or large dogs. In this study, the authors used a nutritive medium previously adapted for piglet fermentation from another medium initially developed for rumen bacteria maintenance without any modification in relation to dog digestion (Williams et al., 2005). The main disadvantage of this model is that it uses digestive fluids collected from dogs to inoculate the vessels, and the inter-individual variability associated with such an approach. Only one static batch model integrates mucin-covered microcosms in order to represent the intestinal mucus, but the associated results are not discussed (Oba et al., 2020). To conclude on these batch models, apart for temperature, fecal inoculation and anaerobiosis, parameters were generally not adapted to canine digestion. Even if *in vitro/in vivo* correlations were established for some of these models, *in vitro* digestion conditions remained far from *in vivo* complexity, and nutrient composition of dog digestive fluids, digestive secretions, or pH variations between small and large intestinal compartments and residence time in each gut section were not considered.

The only dynamic *in vitro* model of the lower dog gut, the SCIME model (for simulation of canine intestinal microbial ecosystem), was adapted from the SHIME system, first set up to reproduce human conditions. The SCIME model is currently unique in reproducing the entire canine GIT from the stomach to the large intestine (Duysburgh et al., 2020). SCIME is composed of four bioreactors simulating the stomach, small intestine, and proximal and distal colon. Only colonic compartments are inoculated with fecal samples from medium-sized dogs. *In vivo* parameters that are reproduced include body temperature, regionalized gastric and intestinal pH, gastrointestinal transit time, digestive secretions (pancreatic juice and bile), and anaerobiosis. The authors mention that most of the parameters were adapted to medium-sized dog digestion, though associated *in vivo* data are not clearly mentioned in the publication. SCIME was validated by comparison with *in vivo* data from ten Beagles digesting fructooligosaccharides regarding microbial composition and SCFA/BCFA production. Of note, *in vitro* results in proximal and distal colon (even if clearly distinct profiles were obtained) were only compared to *in vivo* data in fecal samples. Recently,



Tab. 1: Main *in vitro* models developed to simulate the gastrointestinal tract of dogs and their characteristics
 CO₂, carbon dioxide; FIDO, Functional gastrointestinal DOG model; GET, gastric emptying time; GI, gastrointestinal; LITT, large intestine transit time; N₂, nitrogen; N/A, not applicable; ND, not defined; M-SCIME, Mucosal Simulator of the Canine Intestinal Microbial Ecosystem; SITT, small intestine transit time

References	Dog's generalities		GI compartment modeled	Adaptations from bibliographic research	Validation processes	Temperature	Agitation	pH		Transit time	Food reproduction	Digestive secretions	Ab-sorption	Microbiota																				
	Age (years)	Dog's size						pH kinetic	Value					Presence of microbiota	Process type	Anaero-biosis	Mucus																	
UPPER TRACT																																		
Hervera et al., 2007	ND	ND	Stomach and small intestine	But no reference to <i>in vivo</i> data	<i>In vivo</i> digestibility trial (n=54)	39°C	Magnetic bar	No	Stomach: 2 Small intestine: 6.8	No	Crushed dry extruded canine food	Gastric digestion: pepsin Intestinal digestion: pancreatin	No	No	N/A	No																		
																	MONO-COMPARTMENTAL																	
																	STATIC																	
Lee et al., 2017 Artificial Stomach-Duodenum	ND	Medium	Stomach and duodenum	Adapted from Smeets-Peeters et al., 1998; Carino et al., 2006; Bhattachar et al., 2011	<i>In vivo</i> pharma trial (n=6)	37°C	Paddle	No	Stomach: 6.8 Duodenum: 6.8-7	No	No	Pancreatin solution	No	No	N/A	No																		
																	MULTI-COMPARTMENTAL																	
																	DYNAMIC																	
Smeets-Peeters et al., 1999 FIDO	"Adult"	Medium	Stomach, duodenum, jejunum and ileum	Adapted from Smeets-Peeters et al., 1998	<i>In vivo</i> digestibility trial (5 ileal cannulated dogs)	37°C	Water pressure on flexible wall	Yes	Stomach: 2-6 Duodenum: 6.2 ± 0.2 Jejunum: 6.5 ± 0.2 Ileum: 7.0 ± 0.2	Yes	Crushed dry or canned dog food	Gastric juice, pancreatic juice, intestinal electrolyte solution, bile solution	Passive	No	N/A	No																		
																	MONO-COMPARTMENTAL																	
																	STATIC																	
LOWER TRACT																																		
Vierbaum et al., 2019	4.3 ± 0.9	Medium	Large intestine		No validation	37°C	Continuous shaking at 370 rpm	No	N/A	No	No dog food: Pre-reduced anaerobe sterilized medium	No	No	Yes, from feces	Batch 24 h	Yes	No																	
																		MONO-COMPARTMENTAL																
																		STATIC																
Cutrignelli et al., 2009	3	Large	Large intestine		Based on a literature review	39°C	No	N/A	N/A	No	No	No	No	Yes, from feces	Batch 48 h	Yes	No																	
																		MONO-COMPARTMENTAL																
																		STATIC																
Panasevich et al., 2013	ND	ND	Large intestine	But no reference to <i>in vivo</i> data	<i>In vivo</i> digestibility trial (n=10)	39°C	Periodic mixing	No	N/A	No	No dog food: Anaerobe medium	No	No	Yes, from feces	Batch 12 h	Yes	CO ₂ -gas	No																
																			MONO-COMPARTMENTAL															
																			STATIC															
Sunvold et al., 1995	5-6	Medium	Large intestine		<i>In vivo</i> fermentation study (n=30)	39°C	No	N/A	N/A	No	Nutritive medium based on several solutions of vitamins, oligo-elements, supplemented with fibrous substrates	No	No	Yes, from feces	Batch 24 h	Yes	CO ₂ -gas	No																
																			MONO-COMPARTMENTAL															
																			STATIC															
Van den Abbeele et al., 2020a	ND	ND	Large intestine		Based on a literature review	39°C	Magnetic bar	No	N/A	No	No dog food: Colonic background medium	No	No	Yes, from feces	Batch 48 h	Yes	N ₂ -gas flow	No																
																			MONO-COMPARTMENTAL															
																			STATIC															



No dog food. Colonic background medium	No	6.8	No	Magnetic bar	37°C	No validation	⊗	Large intestine	ND	ND	Large intestine	⊗	No validation	37°C	Continuous shaking at 90 rpm	No	No	No	No dog food. Colonic background medium	No	Batch 24 h	Yes N ₂ -gas flow	No	
No dog food. Filtered digestive fluids added to a nutritive medium previously adapted for rumen bacteria growth	No	N/A	No	Continuous shaking at 90 rpm	39°C	No validation	⊗	Large intestine	4.6	Adult and mature	Large intestine	⊗	No validation	39°C	Continuous shaking at 90 rpm	No	No	No	Colonic background medium	No	Batch 48 h	Yes N ₂ -gas flow	Mucin-covered microcosms	
Dog food added at 9 g/L in stomach juice. Small and large intestine effluents simulated by addition of digested suspension from the previous compartment	Residence times GET: 1 h SITT: 4 h LITT: 6 h (transverse) 10 h (distal)	Stomach: 2 Small intestine: 6.8 Proximal colon: 5.6-5.9 Distal colon: 6.65-6.9	No	Magnetic bar	39°C	SCIME <i>In vivo</i> fermentation study (n=10) and literature M-SCIME Comparison with SCIME results Comparison of <i>in vitro</i> results with fecal inoculum (microbiota)	⊙ But no reference to <i>in vivo</i> data	Stomach, small intestine, proximal colon and distal colon	Medium	ND	ND	Stomach, small intestine, proximal colon and distal colon	⊙ But no reference to <i>in vivo</i> data	Comparison of <i>in vitro</i> results with fecal inoculum (microbiota)	39°C	Magnetic bar	No	No	No	No dog food. Filtered digestive fluids added to a nutritive medium previously adapted for rumen bacteria growth	No	Batch 72 h	Yes CO ₂ -gas	Mucin-covered microcosms
Pancreatic juice and bile	Passive (feed)	Yes, from feces, in the large intestinal compartments only	Continuous	Yes N ₂ -gas flow	Mucin-covered microcosms																			
DYNAMIC												MULTI-COMPARTMENTAL												
Tzortzis et al., 2004	Oba et al., 2020	Bosch et al., 2008	Duysburgh et al., 2020 SCIME	Verstrepen et al., 2021 M-SCIME																				

SCIME was optimized by the addition of mucin-covered plastic beads, based on Mucosal-SHIME (M-SHIME) technology (Van den Abbeele et al., 2009), to reproduce the luminal and mucosal microenvironments of the canine colon. The resulting model was called M-SCIME (Van den Abbeele et al., 2020b; Verstrepen et al., 2021). Due to a lack of *in vivo* data, this optimization was not validated compared to bacterial mucosal profiles in dogs, but only compared to previous results obtained in the M-SHIME. Once again, *in vitro* colonic pH varies widely between currently available models, which could be explained by the fact that authors generally base their model settings on a unique *in vivo* study, whereas large inter-individual variations are observed in dogs depending on age, size, and breed.

6 *In vitro* gut models as powerful tools to study canine digestion

6.1 Scientific and technical challenges to be addressed

As described above, *in vitro* models have not been fully optimized, probably due to the paucity of information on the relevant parameters in dogs. Therefore, many scientific and technical challenges still need to be addressed to improve models of canine digestion and reflect the complexity of this environment (Fig. 4).

First, technical improvements should be considered to simulate canine digestive conditions in each part of the GIT more realistically. Currently, there is no canine chewing simulator, but such a development is not a priority, since most dogs do not chew but swallow large pieces of food. Regarding the upper gut, the FIDO model already shows a high level of complexity. The M-SCIME also possesses a gastric compartment that would merit improvements such as progressive acidification of the chyme (already introduced in the M-SHIME). This change in gastric pH during canine digestion is a key parameter in food disruption and digestion as it influences gastric pepsin and lipase activities (Carrière et al., 1993; Sams et al., 2016). FIDO and M-SCIME both require homogenization of food before *in vitro* digestion. In dogs, larger food particle sizes seem to reach the stomach. Even if little data on this in dogs is available (unlike in humans), some canine studies showed no correlation between food size and density of particles on gastric emptying time or on the entire upper gut digestion process (Gruber et al., 1987; Meyer et al., 1988; Chen et al., 2008; De Cuyper et al., 2018), while others observed that gastric half emptying time increased with higher meal viscosity and fat content (Ehrlein and Pröve, 1982; Palerme et al., 2020). Recently, a new human gastric and small intestinal model, the Engineered Stomach and Small Intestine (ESIN), was developed to fill this gap and handle both ingested liquids and real-size food particles better in simulated digestion studies (Guerra et al., 2016). Even if this model was primarily set up for human applications, it could easily be adapted to reproduce dog digestion.

Another main issue in upper gut models is the use of porcine, bovine, or fungal secretions/enzymes instead of canine ones. Due to ethical constraints, digestive secretions cannot be collected from dogs for this purpose (and are not commercially avail-

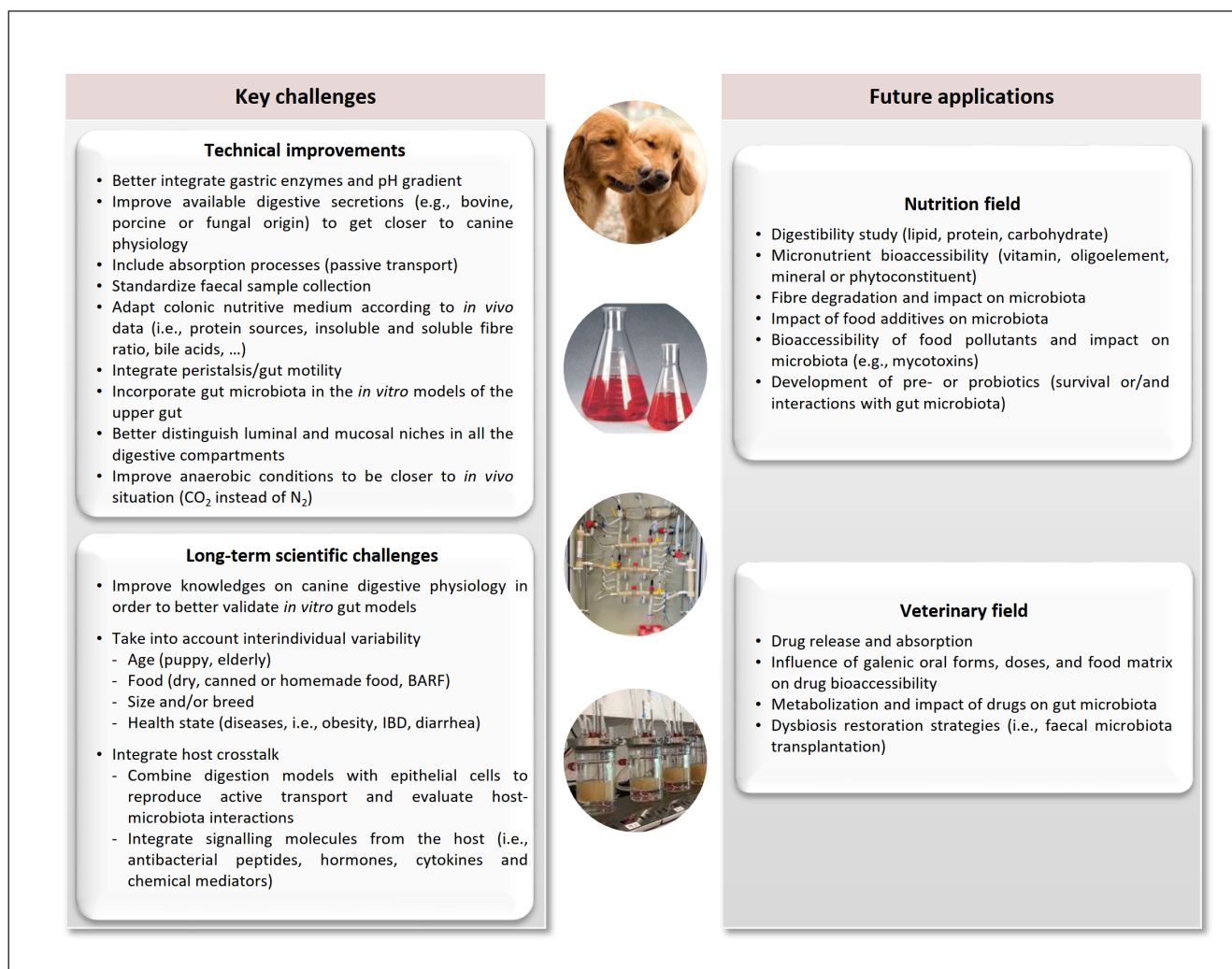


Fig. 4: Main challenges in the development of *in vitro* gut models of the canine digestive tract and their applications in nutritional and veterinary fields

Overview of the main technical and scientific challenges and applications of canine *in vitro* gut models as reliable tools to test or develop new products in the food and pharma fields.

able). Therefore, further investigations are needed to ensure that digestive secretions from other sources are suitable to represent canine ones (in terms of composition and enzymatic activities) to ensure relevant *in vitro* simulation. For example, bile composition and conjugation profiles differ widely between species (Thakare et al., 2018a,b). Thus, further analysis is needed to determine whether porcine bile (largely used in *in vitro* models) is a good model of canine bile (Alvaro et al., 1986).

Another main limitation of current upper gut models is their inability to accurately mimic gastric and intestinal peristalsis. In M-SCIME, peristaltic mixing is reproduced using magnetic stirrers, which is far from the *in vivo* situation. FIDO more closely reproduces peristaltic movements through gentle mixing with pressurized water jackets, but peristaltic force and frequency have not been adapted for dog models, whereas *in vivo* data indicate clear differences between humans and dogs (Boscan et

al., 2013; Warrit et al., 2017; Farmer et al., 2018). Efforts should therefore be invested to more accurately simulate mechanical deformation resulting from peristalsis in dogs, based on what has already been done in human gastric models (Kong and Singh, 2010; Li et al., 2019).

Regarding nutrient absorption in the small intestine, FIDO incorporates dialysis fiber modules in the jejunal and ileal compartments. In a very recent publication on the M-SCIME (Verstrepen et al., 2021), a dialysis step of ground dog feed was integrated to simulate small intestinal absorption by removing high-molecular weight proteins. Adding such dialysis devices to other upper gut models would be beneficial to better represent the *in vivo* situation, even if dialysis modules only reproduce passive absorption of small molecules (e.g., fatty acids, oligo- and monosaccharides, small peptides, amino acids, but also drugs and chemicals) and water. Reproducing passive absorption also ensures bile re-

absorption in the distal intestine and, therefore, a decrease in bile concentrations from the duodenal to the ileal compartments, which is a key process in both dog and human gut physiology.

To further reproduce absorption phenomena and integrate active transport, canine dog models of the upper gut should be coupled with immortalized canine intestinal epithelial cells (cIEC) (Farquhar et al., 2018), as done in human models with TIM-1 and Caco-2 cells (Déat et al., 2009; Bahrami et al., 2011) or with organoids generated from canine duodenal, jejunal and colonic biopsies (Kramer et al., 2020).

A last key point to be raised is the lack of intestinal microbiota in all the upper gut models, whereas bacterial concentrations up to 10^7 CFU/g can be found in the distal small intestine of dogs (Benno et al., 1992; Mentula et al., 2005). Even if the role of intestinal microbiota is poorly defined in dogs (Hooda et al., 2012; Deng and Swanson, 2015; Enright et al., 2016; Mondo et al., 2019; Pilla and Suchodolski, 2020), we can assume that intestinal microbiota is involved at least in carbohydrate digestion and may exert a barrier effect against enteric pathogens as observed in humans (Andoh, 2016). This is even more important since, unlike in humans, an appreciable fraction of dietary fibers seems to be degraded in the canine small intestine (Bednar et al., 2000).

This microbial component of the canine digestive tract is integrated in *in vitro* colon models by inoculation with fecal samples. However, a major issue of all available *in vitro* colon models is that the nutritive medium used to maintain bacterial growth and activity was not adapted to adequately mimic the composition of ileal effluents entering the colon *in vivo*. Using digestive fluids as previously done by Bosch et al. (2008) is not a simple and sustainable solution as it makes *in vitro* models strongly dependent of unstandardized and poorly available *in vivo* samples. Therefore, efforts are still required to better define a growth medium composition based on available data on dog diet, but also protein, lipid and carbohydrate ileal digestibility (Bednar et al., 2000; Flickinger et al., 2003; Propst et al., 2003). It is also important to consider relevant BA concentrations and profiles reaching the colon, as they may shape gut microbiota composition (Ridlon et al., 2014).

All available *in vitro* colonic models are flushed with nitrogen or carbon dioxide to maintain anaerobic conditions inside vessels or bioreactors, which is unlike the *in vivo* situation. In the human Artificial Colon (ARCOL) model, anaerobiosis is maintained solely by the activity of resident microbiota to reflect *in vivo* gut physiology (Friedman et al., 2018; Deschamps et al., 2020; Verdier et al., 2021). Such an adaptation could further increase the relevance of *in vitro* canine colon models.

Moreover, only bacteria have been followed to date as the main component of gut microbiota. Given the importance of other constituents, such as fungi, methanogenic archaea, or even phages or viruses in gut physiology (Barko et al., 2018), it would be of great interest to extend microbial sequencing to these populations.

Lastly, as mentioned for upper gut models, to further investigate host-microbiome crosstalk, canine *in vitro* models should be coupled to cell culture assays, as previously described for human models (Bahrami et al., 2011; Chassaing et al., 2017; Geirnaert et al., 2017; Defois et al., 2018).

Of interest, the Host Microbiota Interaction Module already coupled to the SHIME system (Marzorati et al., 2014) integrates microaerobiosis in close proximity to the epithelium, shaping mucus-associated gut microbiota. This may also be of relevance for the dog colonic ecosystem.

6.2 Future developments and quality requirements

Most available *in vitro* models, and especially the more complex FIDO and M-SCIME systems, have been designed to reproduce a medium-sized dog's digestive conditions when ingesting dry food. As widely described, canine digestion is impacted by body weight or breed (Kendall et al., 1983; Bourreau et al., 2004; Herriot et al., 2005; Weber, 2006; Weber et al., 2017). Considering the influence of body weight and/or breed on canine digestive physicochemical and microbial parameters is undoubtedly a promising line of research to develop more relevant *in vitro* models.

Further, adaptations regarding dog diet (e.g., dry, canned or homemade food, BARF) and age (puppies, adults and elderly dogs) would also bring substantial added value to *in vitro* canine gut simulation. This has been already performed with success in *in vitro* human models (Blanquet et al., 2004; Fehlbaum et al., 2015; Denis et al., 2016; Roussel et al., 2016, 2020; Bondué et al., 2020).

Moving forward, we can foresee the development of *in vitro* gut models simulating not only physiological but also diseased situations, such as those associated with obesity or IBD, as previously done in humans (Bussolo de Souza et al., 2014). In this case, the main objective would be to maintain gut microbiota dysbiosis (considered a typical feature of these pathologies) inside the *in vitro* models. For this purpose, small intestinal and/or colonic models could be inoculated with feces collected from dogs suffering from obesity or IBD, but also all the associated gut parameters, such as the composition of digestive effluents (including bile profiles), pH and retention time should be modified to fulfil specific diseased conditions.

All these technical and scientific improvements would be possible if corresponding canine *in vivo* data were available in the literature (or provided by clinical trials), both for setting-up *in vitro* models and for the validation of their robustness through strong *in vivo-in vitro* correlations. Such validation is necessary to convince future users of the relevance of *in vitro* gut models but is unfortunately rarely performed due to cost, time, or technical limitations.

To date, main gaps in *in vivo* data concern digestive secretions in the upper gut as well as microbial profiles along the digestive tract and characterization of mucus-associated bacteria. Recent developments of non-invasive methods to follow digestive parameters, such as pH, motility, or transit time (wireless motility capsules, e.g., SmartPill) in dogs (Warritt et al., 2017) and in humans (Schwizer et al., 2002; Wang et al., 2005; Zhang et al., 2014) open new avenues and may help to fill these scientific and technological gaps. Regarding such *in vivo* reference studies, it would be of high importance to standardize the experimental conditions in terms of age, breed, weight, but also diet and lifestyle, which all impact digestive parameters (Mahar et al., 2012; Panasevich et al., 2015; Apper et al., 2020; Pilla and Such-



odolski, 2020; You and Kim, 2021). It is also important to exclude breeds showing well-known specific digestive particularities (like German Shepherd) or specific energy needs (like Husky, Great Danes or Terriers) (Zentek and Meyer, 1995) from such *in vivo* assays.

Standardization of procedures is also of concern for *in vitro* studies, and some important guidance is available in the guidance document on Good *In Vitro* Method Practice (GIVIMP) (OECD, 2018), especially for cell culture practices.

In addition to robustness, canine *in vitro* model development involves other quality requirements, such as reproducibility and transferability. Reproducibility is considered a key advantage of *in vitro* studies over *in vivo* experiments and should be systematically assessed. Transferability of canine *in vitro* gut models is hampered by their current early stage of development. The simple *in vitro* systems (such as batch models) can be more easily shared between laboratories than the more complex ones, which require specific technical expertise. Transferability of canine gut models could be accelerated by exchanges between international experts in artificial digestion, as previously done in the frame of the INFOGEST network, which led to the harmonization of static and semi-dynamic *in vitro* protocols in humans (Minekus et al., 2014; Brodtkorb et al., 2019).

6.3 Potential applications of canine gut models in food and pharma

In vitro models represent a powerful platform to study the fate of food and veterinary products in the canine digestive environment, to help elucidate their mechanisms of action and promote innovation in these fields. As parameters can be adjusted in terms of food matrix, age, dog breed or body size, but also by mimicking physiological or pathological situations, we can imagine numerous applications of these systems once developed and validated (Fig. 4). They could provide valuable information to promote the evolution of products already on the market (generic, range extension, etc.) and/or inform the development of new products such as specialized food or innovative drugs. All types of *in vitro* gut models, from the simplest to the more complex ones, can be useful. Static mono-compartmental models are ideal tools to perform pre-screening of many compounds due to their low cost and easy manipulation. Dynamic multi-compartmental systems, which are more expensive and require expertise but reproduce more physiological digestive conditions, can be applied for a more focused study of selected candidate compounds. The applications already performed under human conditions (Souliman et al., 2006; Cordonnier et al., 2015; Lyng et al., 2016; Bianchi et al., 2019; Blancquaert et al., 2019; Kubbinga et al., 2019) in food and pharma fields can inspire similar studies in canine *in vitro* gut models.

To date, *in vitro* models simulating the canine digestive environment have only been used for nutritional applications, mainly to assess fiber digestibility (Sunvold et al., 1995c; Bosch et al., 2008; Cutrignelli et al., 2009; Musco et al., 2018; Van den Abbeele et al., 2020a,b) (Tab. 2). Other applications include evaluation of protein (Kim et al., 2021) or lipid digestibility and bioaccessibility of micronutrients such as vitamins, minerals (van

Zelst et al., 2015; Lee et al., 2017) or phytoconstituents like polyphenols. In addition to digestibility, *in vitro* models can provide valuable information on the effects of fiber on gut microbial composition and activity, through SCFA/BCFA, ammonia or gas measurements (Bosch et al., 2008; Cutrignelli et al., 2009; Van den Abbeele et al., 2020a). This will help to assess the prebiotic status of soluble fibers. In addition to prebiotics, probiotic strains can be tested in the *in vitro* models to evaluate their interactions with gut microbiota (Ogué-Bon et al., 2011) but also their survival (and the effect of mode of administration) and their production of active compounds such as bacteriocins.

Regarding veterinary applications, the kinetics of drug release and absorption can be monitored all along the GIT, as well as the influence of oral formulations (Lee et al., 2017), different doses, fed or fasted state, and food matrix (food-drug interactions). Currently, drug posology is only established based on dog body weight or metabolic weight, but many associated variations in digestive parameters that influence drug bioaccessibility should be considered (Oswald et al., 2015). In addition, *in vitro* gut models can enhance knowledge of drug metabolism by gut microbiota and/or effects of drugs on gut microbiota composition and function (Sjögren et al., 2014). Hence, gut microbial dysbiosis induced by oral antibiotherapy (El Hage et al., 2019) can be simulated in colonic models and further applied to evaluate the efficiency of prebiotics and/or probiotics in restoring microbiota eubiosis.

Lastly, *in vitro* models can be employed to assess the efficiency of new microbial restoration therapy strategies such as fecal microbiota transplantation in a safe way before using animals. Fecal microbiota transplantation is based on the observation that transfer of intestinal contents from a healthy donor to a diseased one can improve gut health. This therapy has been recently tested in dogs for the treatment of post-weaning diarrhea, acute diarrhea, IBD, chronic enteropathies, or parvovirus infections and seems to be promising (Burton et al., 2016; Pereira et al., 2018; Niina et al., 2019; Chaitman and Gaschen, 2020; Chaitman et al., 2020).

7 Conclusion

Canine digestion is a complex and regionalized process involving physicochemical, mechanical, and microbial processes. It plays a central role in maintaining dog health and is increasingly recognized as part of the etiology of intestinal and extra-digestive diseases (such as IBD and obesity) in relation to gut microbiota dysbiosis. When testing or developing new products, food and veterinary industries need to consider these multi-faceted aspects of canine digestion and would benefit from relevant *in vitro* gut models for in-depth mechanistic studies as an alternative to *in vivo* assays.

Up to now, only a restricted number of *in vitro* models has been developed to simulate the canine upper or lower digestive tract. These devices show various levels of complexity, i.e., from static mono-compartmental to dynamic multi-compartmental models. The *in vitro* parameters have not yet been fully



Tab. 2: Application studies of the currently available canine *in vitro* gut models

BCFA, branched-chain fatty acids; GIT, gastro-intestinal tract; SCFA, short-chain fatty acids

References	Applications					Aim of the study
	Macronutrients	Micronutrients	Fibers/Prebiotics	Probiotics	Drugs	
Upper GIT models						
Smeets-Peeters et al., 1999	✓	✓				Effects of small intestinal transit time on protein digestibility and calcium availability from canned dog food
Hervera et al., 2007	✓					<i>In vitro</i> percentage of organic matter disappearance used as a predictor of apparent organic matter and energy digestibility of extruded dog food
van Zelst et al., 2015		✓				Identification of dietary factors that affect selenium accessibility in commercial petfood
Lee et al., 2017					✓	Evaluation of <i>in vitro</i> dissolution performance of five formulations of an acidic BCS Class II compound
Penazzi et al., 2021	✓					Effect of supplementation with black soldier fly (<i>Hermetia illucens</i>) larvae meal in extruded dog food on <i>in vivo</i> and <i>in vitro</i> digestibility
Kim et al., 2021	✓					Effect of thermal processing on ileal digestibility of dry matter and crude protein from raw chicken meat
Lower GIT models						
Sunvold et al., 1995b			✓			Effects of cellulose, beet pulp, citrus pulp, and citrus pectin on microbiota fermentation (organic matter disappearance, SCFA, and lactate)
Sunvold et al., 1995a			✓			<i>In vitro</i> fermentation of selected fibrous substrates: influence of diet composition on substrate organic matter disappearance and SCFA production
Sunvold et al., 1995c			✓			<i>In vitro</i> fermentation of selected fiber sources and metabolism of fiber-supplemented diets
Swanson et al., 2001			✓			Fermentation of vegetable and fruit fiber sources compared to fiber standards following SCFA production, organic matter disappearance, and gas production
Tzortzis et al., 2004			✓			Fermentation properties of galactooligosaccharides synthesized by α-galactosidase from <i>Lactobacillus reuteri</i> (SCFA)
Biagi et al., 2008			✓			Effects of fiber sources on microbiota composition and activity (SCFA, ammonia, and gas)
Bosch et al., 2008			✓			Fermentation kinetics of fibers from canine foods (gas and SCFA)
Cutrignelli et al., 2009			✓			Impact of different carbohydrate sources on microbiota fermentation (gas, SCFA, BCFA, ammonia)
Ogué-Bon et al., 2010 (39°C)			✓	✓		Evaluation of a symbiotic combination on probiotic growth and microbiota activity (SCFA)
Ogué-Bon et al., 2011 (39°C)			✓	✓		Effects of rice bran combined with <i>Lactobacillus acidophilus</i> and <i>Bifidobacterium longum</i> on microbiota activity (SCFA)
Panasevich et al., 2013			✓			Characterization of potato fiber fermentability by canine microbiota (SCFA and BCFA)
Panasevich et al., 2015			✓			Effect of soluble corn fibers on nitrogen-corrected true metabolizable energy and fermentability by microbiota (SCFA)
Vierbaum et al., 2019			✓			Effects of <i>Yucca schidigera</i> powder and inulin on protein fermentation metabolites (SCFA, BCFA, phenols and indoles, biogenic amines, ammonia)
Donadelli et al., 2019			✓			Effects of different fiber sources used in petfood on organic matter disappearance and SCFA/BCFA production
Oba et al., 2020			✓			Potential fermentation and prebiotic effects of GNU100, an animal milk oligosaccharide biosimilar, on microbial communities and metabolite production (gas, SCFA, BCFA, lactate)
Van den Abbeele et al., 2020a			✓			Effects of yeast-derived formulation on microbial composition and activity (gas, SCFA, ammonium)
Traughber et al., 2020			✓			Fermentability of legumes by microbiota and metabolites production (SCFA, BCFA and gas)
Duysburgh et al., 2020			✓			Effect of fructooligosaccharide supplementation on microbial community composition and activity (SCFA, BCFA and ammonium)
Van den Abbeele et al., 2020b			✓			Effect of a <i>S. cerevisiae</i> -based product on microbiota composition and production of fermentation metabolites (SCFA, BCFA and lactate)



adapted to *in vivo* canine digestion, and some of the models are not yet validated for their application, mainly due to a paucity of *in vivo* data. Therefore, many scientific and technical challenges must be overcome to optimize canine models to represent canine digestive physiology in order to realize their potential in food and pharma studies, e.g., by simulating specific digestive conditions associated with different dog sizes, breeds, or ages, under healthy or diseased conditions.

References

- Akimoto, M., Nagahata, N., Furuya, A. et al. (2000). Gastric pH profiles of beagle dogs and their use as an alternative to human testing. *Eur J Pharm Biopharm* 49, 99-102. doi:10.1016/s0939-6411(99)00070-3
- Alexander, C., Guard, B. C., Suchodolski, J. S. et al. (2019). Cholestyramine decreases apparent total tract macronutrient digestibility and alters fecal characteristics and metabolites of healthy adult dogs. *J Anim Sci* 97, 1020-1026. doi:10.1093/jas/sky437
- AlShawaqfeh, M. K., Wajid, B., Minamoto, Y. et al. (2017). A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiol Ecol* 93, fix136. doi:10.1093/femsec/fix136
- Alvaro, D., Cantafora, A., Attili, A. F. et al. (1986). Relationships between bile salts hydrophilicity and phospholipid composition in bile of various animal species. *Comp Biochem Physiol B Comp Biochem* 83, 551-554. doi:10.1016/0305-0491(86)90295-6
- Andoh, A. (2016). Physiological role of gut microbiota for maintaining human health. *Digestion* 93, 176-181. doi:10.1159/000444066
- Apper, E., Privet, L., Taminiau, B. et al. (2020). Relationships between gut microbiota, metabolome, body weight, and glucose homeostasis of obese dogs fed with diets differing in prebiotic and protein content. *Microorganisms* 8, 513. doi:10.3390/microorganisms8040513
- Arnbjerg, J. (1992). Gastric emptying time in the dog and cat. *Food and Agriculture Organization of the United Nations* 28, 77-81.
- Aspinall, V. (2004). Anatomy and physiology of the dog and cat 8. The digestive system. *Veterinary Nursing Journal* 19, 94-99. doi:10.1080/17415349.2004.11013260
- Axelsson, E., Ratnakumar, A., Arendt, M.-L. et al. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495, 360-364. doi:10.1038/nature11837
- Bahrani, B., Child, M. W., Macfarlane, S. et al. (2011). Adherence and cytokine induction in Caco-2 cells by bacterial populations from a three-stage continuous-culture model of the large intestine. *Appl Environ Microbiol* 77, 2934-2942. doi:10.1128/AEM.02244-10
- Barko, P. C., McMichael, M. A., Swanson, K. S. et al. (2018). The gastrointestinal microbiome: A review. *J Vet Intern Med* 32, 9-25. doi:10.1111/jvim.14875
- Barroso, E., Cueva, C., Peláez, C. et al. (2015). The computer-controlled multicompartimental dynamic model of the gastrointestinal system SIMGI. In K. Verhoeckx, P. Cotter, I. López-Expósito et al. (eds.), *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*. Cham, Switzerland: Springer.
- Bednar, G. E., Murray, S. M., Patil, A. R. et al. (2000). Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. *Arch Tierernahr* 53, 127-140. doi:10.1080/17450390009381942
- Beloshapka, A. N., Wolff, A. K. and Swanson, K. S. (2012). Effects of feeding polydextrose on faecal characteristics, microbiota and fermentative end products in healthy adult dogs. *Br J Nutr* 108, 638-644. doi:10.1017/S0007114511005927
- Benno, Y., Nakao, H., Uchida, K. et al. (1992). Impact of the advances in age on the gastrointestinal microflora of beagle dogs. *J Vet Med Sci* 54, 703-706. doi:10.1292/jvms.54.703
- Bermudez Sanchez, S., Pilla, R., Sarawichitr, B. et al. (2020). Fecal microbiota in client-owned obese dogs changes after weight loss with a high-fiber-high-protein diet. *PeerJ* 8, e9706. doi:10.7717/peerj.9706
- Biagi, G., Cipollini, I. and Zaghini, G. (2008). In vitro fermentation of different sources of soluble fiber by dog faecal inoculum. *Vet Res Commun* 32, 335-337. doi:10.1007/s11259-008-9142-y
- Bianchi, F., Lopes, N. P., Adorno, M. A. T. et al. (2019). Impact of combining acerola by-product with a probiotic strain on a gut microbiome model. *Int J Food Sci Nutr* 70, 182-194. doi:10.1080/09637486.2018.1498065
- Bickel, M. and Kauffman, G. L. (1981). Gastric gel mucus thickness: Effect of distention, 16,16-dimethyl prostaglandin e₂, and carbenoxolone. *Gastroenterology* 80, 770-775.
- Blake, A. B., Guard, B. C., Honneffer, J. B. et al. (2019). Altered microbiota, fecal lactate, and fecal bile acids in dogs with gastrointestinal disease. *PLoS One* 14, e0224454. doi:10.1371/journal.pone.0224454
- Blancquaert, L., Vervaeke, C. and Derave, W. (2019). Predicting and testing bioavailability of magnesium supplements. *Nutrients* 11, 1663. doi:10.3390/nu11071663
- Blanquet, S., Zeijdner, E., Beyssac, E. et al. (2004). A dynamic artificial gastrointestinal system for studying the behavior of orally administered drug dosage forms under various physiological conditions. *Pharm Res* 21, 585-591. doi:10.1023/b:pham.0000022404.70478.4b
- Boillat, C. S., Gaschen, F. P. and Hosgood, G. L. (2010). Assessment of the relationship between body weight and gastrointestinal transit times measured by use of a wireless motility capsule system in dogs. *Am J Vet Res* 71, 898-902. doi:10.2460/ajvr.71.8.898
- Bondue, P., Lebrun, S., Taminiau, B. et al. (2020). A toddler SHIME® model to study microbiota of young children. *FEMS Microbiol Lett* 367, fnaa135. doi:10.1093/femsle/fnaa135
- Boscan, P., Cochran, S., Monnet, E. et al. (2013). Effect of prolonged general anesthesia with sevoflurane and laparoscopic surgery on gastric and small bowel propulsive motility and pH in dogs. *Vet Anaesth Analg* 41, 73-81. doi:10.1111/vaa.12093

- Bosch, G., Pellikaan, W. F., Rutten, P. G. P. et al. (2008). Comparative in vitro fermentation activity in the canine distal gastrointestinal tract and fermentation kinetics of fiber sources. *J Anim Sci* 86, 2979-2989. doi:10.2527/jas.2007-0819
- Bourreau, J., Hernot, D., Bailhache, E. et al. (2004). Gastric emptying rate is inversely related to body weight in dog breeds of different sizes. *J Nutr* 134, 2039S-2041S. doi:10.1093/jn/134.8.2039S
- Brodkorb, A., Egger, L., Alminger, M. et al. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nat Protoc* 14, 991-1014. doi:10.1038/s41596-018-0119-1
- Burton, E. N., O'Connor, E., Ericsson, A. C. et al. (2016). Evaluation of fecal microbiota transfer as treatment for postweaning diarrhea in research-colony puppies. *J Am Assoc Lab Anim Sci* 55, 582-587.
- Bussolo de Souza, C., Roeselers, G., Troost, F. et al. (2014). Prebiotic effects of cassava bagasse in TNO's in vitro model of the colon in lean versus obese microbiota. *J Funct Foods* 11, 210-220. doi:10.1016/j.jff.2014.09.019
- Carrière, F., Laugier, R., Barrowman, J. A. et al. (1993). Gastric and pancreatic lipase levels during a test meal in dogs. *Scand J Gastroenterol* 28, 443-454. doi:10.3109/00365529309098247
- Cassmann, E., White, R., Atherly, T. et al. (2016). Alterations of the ileal and colonic mucosal microbiota in canine chronic enteropathies. *PLoS One* 11, e0147321. doi:10.1371/journal.pone.0147321
- Chaitman, J. and Gaschen, F. (2020). Fecal microbiota transplantation in dogs. *Vet Clin North Am Small Anim Pract* 51, 219-223. doi:10.1016/j.cvsm.2020.09.012
- Chaitman, J., Ziese, A.-L., Pilla, R. et al. (2020). Fecal microbial and metabolic profiles in dogs with acute diarrhea receiving either fecal microbiota transplantation or oral metronidazole. *Front Vet Sci* 7, 192. doi:10.3389/fvets.2020.00192
- Chassaing, B., Van de Wiele, T., De Bodt, J. et al. (2017). Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation. *Gut* 66, 1414-1427. doi:10.1136/gutjnl-2016-313099
- Chen, E. P., Mahar Doan, K. M., Portelli, S. et al. (2008). Gastric pH and gastric residence time in fasted and fed conscious cynomolgus monkeys using the Bravo pH system. *Pharm Res* 25, 123-134. doi:10.1007/s11095-007-9358-5
- Chèvreton, P. (2018). *The Gut Microbiota of Domestic Carnivorous: State of Play*. Faculté de Médecine de Créteil.
- Chun, J. L., Ji, S. Y., Lee, S. D. et al. (2020). Difference of gut microbiota composition based on the body condition scores in dogs. *J Anim Sci Technol* 62, 239-246. doi:10.5187/jast.2020.62.2.239
- Cinquin, C., Le Blay, G., Fliss, I. et al. (2006). New three-stage in vitro model for infant colonic fermentation with immobilized fecal microbiota: Model for infant colon fermentation. *FEMS Microbiol Ecol* 57, 324-336. doi:10.1111/j.1574-6941.2006.00117.x
- Collins, S. B., Perez-Camargo, G., Gettinby, G. et al. (2001). Development of a technique for the in vivo assessment of flatulence in dogs. *Am J Vet Res* 62, 1014-1019. doi:10.2460/ajvr.2001.62.1014
- Cordonnier, C., Thévenot, J., Etienne-Mesmin, L. et al. (2015). Dynamic in vitro models of the human gastrointestinal tract as relevant tools to assess the survival of probiotic strains and their interactions with gut microbiota. *Microorganisms* 3, 725-745. doi:10.3390/microorganisms3040725
- Cullen, J. J. and Kelly, K. A. (1996). Functional characteristics of canine pylorus in health, with pyloroplasty, and after pyloric reconstruction. *Dig Dis Sci* 41, 711-719. doi:10.1007/BF02213127
- Cutrignelli, M. I., Bovera, F., Tudisco, R. et al. (2009). In vitro fermentation characteristics of different carbohydrate sources in two dog breeds (German shepherd and Neapolitan mastiff). *J Anim Physiol Anim Nutr* 93, 305-312. doi:10.1111/j.1439-0396.2009.00931.x
- De Cuyper, A., Hesta, M., Tibosch, S. et al. (2018). How does dietary particle size affect carnivore gastrointestinal transit: A dog model. *J Anim Physiol Anim Nutr (Berl)* 102, e615-e622. doi:10.1111/jpn.12803
- Déat, E., Blanquet-Diot, S., Jarrige, J.-F. et al. (2009). Combining the dynamic TNO-gastrointestinal tract system with a Caco-2 cell culture model: Application to the assessment of lycopene and alpha-tocopherol bioavailability from a whole food. *J Agric Food Chem* 57, 11314-11320. doi:10.1021/jf902392a
- Defois, C., Ratel, J., Garrait, G. et al. (2018). Food chemicals disrupt human gut microbiota activity and impact intestinal homeostasis as revealed by in vitro systems. *Sci Rep* 8, 11006. doi:10.1038/s41598-018-29376-9
- Deng, P. and Swanson, K. S. (2015). Gut microbiota of humans, dogs and cats: Current knowledge and future opportunities and challenges. *Br J Nutr* 113, S6-S17. doi:10.1017/S0007114514002943
- Denis, S., Sayd, T., Georges, A. et al. (2016). Digestion of cooked meat proteins is slightly affected by age as assessed using the dynamic gastrointestinal TIM model and mass spectrometry. *Food Funct* 7, 2682-2691. doi:10.1039/C6FO00120C
- Deschamps, C., Fournier, E., Uriot, O. et al. (2020). Comparative methods for fecal sample storage to preserve gut microbial structure and function in an in vitro model of the human colon. *Appl Microbiol Biotechnol* 104, 10233-10247. doi:10.1007/s00253-020-10959-4
- Detweiler, K. B., He, F., Mangian, H. F. et al. (2019). Effects of high inclusion of soybean hulls on apparent total tract macronutrient digestibility, fecal quality, and fecal fermentative end-product concentrations in extruded diets of adult dogs. *J Anim Sci* 97, 1027-1035. doi:10.1093/jas/skz015
- Donadelli, R. A., Titgemeyer, E. C. and Aldrich, C. G. (2019). Organic matter disappearance and production of short- and branched-chain fatty acids from selected fiber sources used in pet foods by a canine in vitro fermentation model. *J Anim Sci* 97, 4532-4539. doi:10.1093/jas/skz302
- Dressman, J. B. (1986). Comparison of canine and human gastrointestinal physiology. *Pharm Res* 3, 123-131. doi:10.1023/A:1016353705970
- Duboc, H., Rajca, S., Rainteau, D. et al. (2013). Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in



- inflammatory bowel diseases. *Gut* 62, 531-539. doi:10.1136/gutjnl-2012-302578
- Dufourny, S., Everaert, N., Lebrun, S. et al. (2019). Baby-SPIME: A dynamic in vitro piglet model mimicking gut microbiota during the weaning process. *J Microbiol Methods* 167, 105735. doi:10.1016/j.mimet.2019.105735
- Durand, A. (2010). *Entéropathies exsudatives chez le chien: Proposition d'une stratégie thérapeutique diététique et médicamenteuse*. Université Claude Bernard.
- Duysburgh, C., Ossieur, W. P., De Paepe, K. et al. (2020). Development and validation of the Simulator of the Canine Intestinal Microbial Ecosystem (SCIME). *J Anim Sci* 98, skz357. doi:10.1093/jas/skz357
- Ehrlein, H.-J. and Pröve, J. (1982). Effect of viscosity of test meals on gastric emptying in dogs. *Exp Physiol* 67, 419-425. doi:10.1113/expphysiol.1982.sp002657
- Eisenhauer, L., Vahjen, W., Dadi, T. et al. (2019). Effects of Brewer's spent grain and carrot pomace on digestibility, fecal microbiota, and fecal and urinary metabolites in dogs fed low- or high-protein diets. *J Anim Sci* 97, 4124-4133. doi:10.1093/jas/skz264
- El Hage, R., Hernandez-Sanabria, E., Calatayud Arroyo, M. et al. (2019). Propionate-producing consortium restores antibiotic-induced dysbiosis in a dynamic in vitro model of the human intestinal microbial ecosystem. *Front Microbiol* 10, 1206. doi:10.3389/fmicb.2019.01206
- Enright, E. F., Gahan, C. G. M., Joyce, S. A. et al. (2016). The impact of the gut microbiota on drug metabolism and clinical outcome. *Yale J Biol Med* 89, 375-382.
- Etienne-Mesmin, L., Chassaing, B., Desvaux, M. et al. (2019). Experimental models to study intestinal microbes-mucus interactions in health and disease. *FEMS Microbiol Rev* 43, 457-489. doi:10.1093/femsre/fuz013
- Farmer, A. D., Wegeberg, A.-M. L., Brock, B. et al. (2018). Regional gastrointestinal contractility parameters using the wireless motility capsule: Inter-observer reproducibility and influence of age, gender and study country. *Aliment Pharmacol Ther* 47, 391-400. doi:10.1111/apt.14438
- Farquhar, M. J., McCluskey, E., Staunton, R. et al. (2018). Characterisation of a canine epithelial cell line for modelling the intestinal barrier. *Altern Lab Anim* 46, 115-132. doi:10.1177/026119291804600304
- Fehlbaum, S., Chassard, C., Haug, M. C. et al. (2015). Design and investigation of PolyFermS in vitro continuous fermentation models inoculated with immobilized fecal microbiota mimicking the elderly colon. *PLoS One* 10, e0142793. doi:10.1371/journal.pone.0142793
- Fitzpatrick, L. R. and Jenabzadeh, P. (2020). IBD and bile acid absorption: Focus on pre-clinical and clinical observations. *Front Physiol* 11, 564. doi:10.3389/fphys.2020.00564
- Fleury, M. A., Le Goff, O., Denis, S. et al. (2017). Development and validation of a new dynamic in vitro model of the piglet colon (PigutIVM): Application to the study of probiotics. *Appl Microbiol Biotechnol* 101, 2533-2547. doi:10.1007/s00253-017-8122-y
- Flickinger, E. A., Schreijen, E. M. W. C., Patil, A. R. et al. (2003). Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. *J Anim Sci* 81, 2008-2018. doi:10.2527/2003.8182008x
- Forster, G. M., Stockman, J., Noyes, N. et al. (2018). A comparative study of serum biochemistry, metabolome and microbiome parameters of clinically healthy, normal weight, overweight, and obese companion dogs. *Top Companion Anim Med* 33, 126-135. doi:10.1053/j.tcam.2018.08.003
- Freiche, V. and Hernandez, J. (2010). *Gastro-entérologie canine et féline: De la clinique à la thérapeutique*. Elsevier Masson.
- Friedman, E. S., Bittinger, K., Esipova, T. V. et al. (2018). Microbes vs. chemistry in the origin of the anaerobic gut lumen. *Proc Natl Acad Sci U S A* 115, 4170-4175. doi:10.1073/pnas.1718635115
- Garcia-Mazcorro, J. F., Dowd, S. E., Poulsen, J. et al. (2012). Abundance and short-term temporal variability of fecal microbiota in healthy dogs. *Microbiologyopen* 1, 340-347. doi:10.1002/mbo3.36
- Garcia-Mazcorro, J. F., Minamoto, Y., Kawas, J. R. et al. (2020). Akkermansia and microbial degradation of mucus in cats and dogs: Implications to the growing worldwide epidemic of pet obesity. *Vet Sci* 7, 44. doi:10.3390/vetsci7020044
- Geirnaert, A., Calatayud, M., Grootaert, C. et al. (2017). Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. *Sci Rep* 7, 11450. doi:10.1038/s41598-017-11734-8
- Gibson, G. R., Cummings, J. H. and Macfarlane, G. T. (1988). Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl Environ Microbiol* 54, 2750-2755. doi:10.1128/AEM.54.11.2750-2755.1988
- Giffard, C. J., Collins, S. B., Stoodley, N. C. et al. (2001). Administration of charcoal, Yucca schidigera, and zinc acetate to reduce malodorous flatulence in dogs. *J Am Vet Med Assoc* 218, 892-896. doi:10.2460/javma.2001.218.892
- Grandjean, D. and Haymann, F. (2010). *Encyclopédie du chien Royal Canin*.
- Gruber, P., Rubinstein, A., Li, V. H. K. et al. (1987). Gastric emptying of nondigestible solids in the fasted dog. *J Pharm Sci* 76, 117-122. doi:10.1002/jps.2600760207
- Guerra, A., Etienne-Mesmin, L., Livrelli, V. et al. (2012). Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends Biotechnol* 30, 591-600. doi:10.1016/j.tibtech.2012.08.001
- Guerra, A., Denis, S., Goff, O. et al. (2016). Development and validation of a new dynamic computer-controlled model of the human stomach and small intestine. *Biotechnol Bioeng* 113, 1325-1335. doi:10.1002/bit.25890
- Gupta, P. K. and Robinson, J. R. (1988). Gastric emptying of liquids in the fasted dog. *Int J Pharm* 43, 45-52. doi:10.1016/0378-5173(88)90057-9
- Hamper, B. (2016). Current topics in canine and feline obesity. *Vet Clin North Am Small Anim Pract* 46, 785-795. doi:10.1016/j.cvsm.2016.04.003

- Handl, S., German, A. J., Holden, S. L. et al. (2013). Faecal microbiota in lean and obese dogs. *FEMS Microbiol Ecol* 84, 332-343. doi:10.1111/1574-6941.12067
- Hernot, D. C., Biourge, V. C., Martin, L. J. et al. (2005). Relationship between total transit time and faecal quality in adult dogs differing in body size. *J Anim Physiol Anim Nutr* 89, 189-193. doi:10.1111/j.1439-0396.2005.00544.x
- Hervera, M., Baucells, M. D., Blanch, F. et al. (2007). Prediction of digestible energy content of extruded dog food by in vitro analyses. *J Anim Physiol Anim Nutr (Berl)* 91, 205-209. doi:10.1111/j.1439-0396.2007.00693.x
- Hinder, R. A. and Kelly, K. A. (1977). Canine gastric emptying of solids and liquids. *Am J Physiol Endocrinol Metab* 233, E335. doi:10.1152/ajpendo.1977.233.4.E335
- Hooda, S., Minamoto, Y., Suchodolski, J. S. et al. (2012). Current state of knowledge: The canine gastrointestinal microbiome. *Anim Health Res Rev* 13, 78-88. doi:10.1017/S1466252312000059
- Hornof, W. J., Koblik, P. D., Strombeck, D. R. et al. (1989). Scintigraphic evaluation of solid-phase gastric emptying in the dog. *Veterinary Radiology* 30, 242-248. doi:10.1111/j.1740-8261.1989.tb01794.x
- Iacopetti, I., Perazzi, A., Badon, T. et al. (2017). Salivary pH, calcium, phosphorus and selected enzymes in healthy dogs: A pilot study. *BMC Vet Res* 13, 330. doi:10.1186/s12917-017-1256-4
- Junginger, J., Lemensieck, F., Moore, P. F. et al. (2014). Canine gut dendritic cells in the steady state and in inflammatory bowel disease. *Innate Immun* 20, 145-160. doi:10.1177/1753425913485475
- Kakimoto, T., Kanemoto, H., Fukushima, K. et al. (2017). Effect of a high-fat-high-cholesterol diet on gallbladder bile acid composition and gallbladder motility in dogs. *Am J Vet Res* 78, 1406-1413. doi:10.2460/ajvr.78.12.1406
- Kalantzi, L., Persson, E., Polentarutti, B. et al. (2006). Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents. *Pharm Res* 23, 1373-1381. doi:10.1007/s11095-006-0207-8
- Kararli, T. T. (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos* 16, 351-380. doi:10.1002/bdd.2510160502
- Kendall, P. T., Blaza, S. E. and Smith, P. M. (1983). Influence of level of intake and dog size on apparent digestibility of dog foods. *Br Vet J* 139, 361-362. doi:10.1016/s0007-1935(17)30442-6
- Kienzle, E. (1988). Enzymaktivität in Pancreas, Darmwand und Chymus des Hundes in Abhängigkeit von Alter und Futterart. (Enzyme activity in pancreatic tissue, intestinal mucosa and chyme of dogs in relation to age and diet). *J Anim Physiol Anim Nutr* 60, 276-288. doi:10.1111/j.1439-0396.1988.tb00203.x
- Kim, H., Jung, A. H., Park, S. H. et al. (2021). In vitro protein disappearance of raw chicken as dog foods decreased by thermal processing, but was unaffected by non-thermal processing. *Animals (Basel)* 11, 1256. doi:10.3390/ani11051256
- Kong, F. and Singh, R. P. (2010). A human gastric simulator (HGS) to study food digestion in human stomach. *J Food Sci* 75, E627-635. doi:10.1111/j.1750-3841.2010.01856.x
- Koziolek, M., Grimm, M., Bollmann, T. et al. (2019). Characterization of the GI transit conditions in Beagle dogs with a telemetric motility capsule. *Eur J Pharm Biopharm* 136, 221-230. doi:10.1016/j.ejpb.2019.01.026
- Kramer, N., Pratscher, B., Meneses, A. M. C. et al. (2020). Generation of differentiating and long-living intestinal organoids reflecting the cellular diversity of canine intestine. *Cells* 9, 822. doi:10.3390/cells9040822
- Kubbinga, M., Augustijns, P., García, M. A. et al. (2019). The effect of chitosan on the bioaccessibility and intestinal permeability of acyclovir. *Eur J Pharm Biopharm* 136, 147-155. doi:10.1016/j.ejpb.2019.01.021
- Larcheveque, S. (2019). *Etat des connaissances sur la muqueuse biliaire chez le chien : Étude bibliographique*. Faculté de Médecine de Créteil.
- Lavy, E., Goldberger, D., Friedman, M. et al. (2012). pH values and mineral content of saliva in different breeds of dogs. *Isr J Vet Med* 67, 244-2448.
- Lee, C.-M., Luner, P. E., Locke, K. et al. (2017). Application of an artificial stomach-duodenum reduced gastric pH dog model for formulation principle assessment and mechanistic performance understanding. *J Pharm Sci* 106, 1987-1997. doi:10.1016/j.xphs.2017.02.015
- Li, Y., Fortner, L. and Kong, F. (2019). Development of a gastric simulation model (GSM) incorporating gastric geometry and peristalsis for food digestion study. *Food Res Int* 125, 108598. doi:10.1016/j.foodres.2019.108598
- Lui, C. Y., Amidon, G. L., Berardi, R. R. et al. (1986). Comparison of gastrointestinal pH in dogs and humans: Implications on the use of the beagle dog as a model for oral absorption in humans. *J Pharm Sci* 75, 271-274. doi:10.1002/jps.2600750313
- Lyng, E., Havenaar, R., Shastri, P. et al. (2016). Increased bioavailability of celecoxib under fed versus fasted conditions is determined by postprandial bile secretion as demonstrated in a dynamic gastrointestinal model. *Drug Dev Ind Pharm* 42, 1334-1339. doi:10.3109/03639045.2015.1135935
- Mahar, K. M., Portelli, S., Coatney, R. et al. (2012). Gastric pH and gastric residence time in fasted and fed conscious Beagle dogs using the Bravo® pH system. *J Pharm Sci* 101, 2439-2448. doi:10.1002/jps.23159
- Malewska, K., Rychlik, A., Nieradka, R. et al. (2011). Treatment of inflammatory bowel disease (IBD) in dogs and cats. *Pol J Vet Sci* 14, 165-171. doi:10.2478/v10181-011-0026-7
- Manchester, A. C., Webb, C. B., Blake, A. B. et al. (2019). Long-term impact of tylosin on fecal microbiota and fecal bile acids of healthy dogs. *J Vet Intern Med* 33, 2605-2617. doi:10.1111/jvim.15635
- Martinez, M. (2002). Applying the biopharmaceutics classification system to veterinary pharmaceutical products Part II. Physiological considerations. *Adv Drug Deliv Rev* 54, 825-850. doi:10.1016/S0169-409X(02)00071-6
- Marzorati, M., Vanhoecke, B., De Ryck, T. et al. (2014). The



- HMITM module: A new tool to study the host-microbiota interaction in the human gastrointestinal tract in vitro. *BMC Microbiol* 14, 133. doi:10.1186/1471-2180-14-133
- Ménard, O., Picque, D. and Dupont, D. (2015). The DIDGI® System. In K. Verhoeckx, P. Cotter, I. López-Expósito et al. (eds.), *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*. Cham, Switzerland: Springer.
- Mentula, S., Harmoinen, J., Heikkilä, M. et al. (2005). Comparison between cultured small-intestinal and fecal microbiotas in beagle dogs. *Appl Environ Microbiol* 71, 4169-4175. doi:10.1128/AEM.71.8.4169-4175.2005
- Meunier, J. P., Manzanilla, E. G., Anguita, M. et al. (2008). Evaluation of a dynamic in vitro model to simulate the porcine ileal digestion of diets differing in carbohydrate composition. *J Anim Sci* 86, 1156-1163. doi:10.2527/jas.2007-0145
- Meyer, J. H., Dressman, J., Fink, A. et al. (1985). Effect of size and density on canine gastric emptying of nondigestible solids. *Gastroenterology* 89, 805-813. doi:10.1016/0016-5085(85)90576-1
- Meyer, J. H., Elashoff, J., Porter-Fink, V. et al. (1988). Human postprandial gastric emptying of 1-3-millimeter spheres. *Gastroenterology* 94, 1315-1325. doi:10.1016/0016-5085(88)90669-5
- Minamoto, Y., Otoni, C. C., Steelman, S. M. et al. (2015). Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic inflammatory bowel disease. *Gut Microbes* 6, 33-47. doi:10.1080/19490976.2014.997612
- Minikus, M., Marteau, P., Havenaar, R. et al. (1995). A multicompartimental dynamic computer-controlled model simulating the stomach and small intestine. *Altern Lab Anim* 23, 197-209. doi:10.1177/026119299502300205
- Minikus, M., Alminger, M., Alvito, P. et al. (2014). A standardised static in vitro digestion method suitable for food – An international consensus. *Food Funct* 5, 1113-1124. doi:10.1039/c3fo60702j
- Molly, K., Vande Woestyne, M. and Verstraete, W. (1993). Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl Microbiol Biotechnol* 39, 254-258. doi:10.1007/bf00228615
- Mondo, E., Marliani, G., Accorsi, P. A. et al. (2019). Role of gut microbiota in dog and cat's health and diseases. *Open Vet J* 9, 253-258. doi:10.4314/ovj.v9i3.10
- Moon, C. D., Young, W., Maclean, P. H. et al. (2018). Metagenomic insights into the roles of Proteobacteria in the gastrointestinal microbiomes of healthy dogs and cats. *MicrobiologyOpen* 7, e00677. doi:10.1002/mbo3.677
- Morris, J. G. and Rogers, G. R. (1989). Comparative aspects of nutrition and metabolism of dogs and cats. In H. Burger and J. P. W. Rivers (eds.), *Nutrition of the Dog and Cat* (35-66). Cambridge, UK: Cambridge University Press.
- Musco, N., Calabrò, S., Roberti, F. et al. (2018). In vitro evaluation of *Saccharomyces cerevisiae* cell wall fermentability using a dog model. *J Anim Physiol Anim Nutr (Berl)* 102, Suppl 1, 24-30. doi:10.1111/jpn.12864
- Nagahara, T., Ohno, K., Kanemoto, H. et al. (2018). Effect of prednisolone administration on gallbladder emptying rate and gallbladder bile composition in dogs. *Am J Vet Res* 79, 1050-1056. doi:10.2460/ajvr.79.10.1050
- Nakayama, F. (1969). Composition of gallstone and bile: Species difference. *J Lab Clin Med* 73, 623-630.
- Niina, A., Kibe, R., Suzuki, R. et al. (2019). Improvement in clinical symptoms and fecal microbiome after fecal microbiota transplantation in a dog with inflammatory bowel disease. *Vet Med (Auckl)* 10, 197-201. doi:10.2147/VMRR.S230862
- Nogueira, J. P. D. S., He, F., Mangian, H. F. et al. (2019). Dietary supplementation of a fiber-prebiotic and saccharin-eugenol blend in extruded diets fed to dogs. *J Anim Sci* 97, 4519-4531. doi:10.1093/jas/skz293
- Oba, P. M., Vidal, S., Wyss, R. et al. (2020). Effect of a novel animal milk oligosaccharide biosimilar on the gut microbial communities and metabolites of in vitro incubations using feline and canine fecal inocula. *J Anim Sci* 98, skaa273. doi:10.1093/jas/skaa273
- OECD (2018). Guidance Document on Good In Vitro Method Practices (GIVIMP). *OECD Series on Testing and Assessment, No. 286*. OECD Publishing, Paris. doi:10.1787/9789264304796-en
- Ogué-Bon, E., Khoo, C., McCartney, A. L. et al. (2010). In vitro effects of synbiotic fermentation on the canine faecal microbiota. *FEMS Microbiol Ecol* 73, 587-600. doi:10.1111/j.1574-6941.2010.00915.x
- Ogué-Bon, E., Khoo, C., Hoyles, L. et al. (2011). In vitro fermentation of rice bran combined with *Lactobacillus acidophilus* 14 150B or *Bifidobacterium longum* 05 by the canine faecal microbiota. *FEMS Microbiol Ecol* 75, 365-376. doi:10.1111/j.1574-6941.2010.01014.x
- Osto, M. and Lutz, T. A. (2015). Translational value of animal models of obesity – Focus on dogs and cats. *Eur J Pharmacol* 759, 240-252. doi:10.1016/j.ejphar.2015.03.036
- Oswald, H., Sharkey, M., Pade, D. et al. (2015). Canine gastrointestinal physiology: Breeds variations that can influence drug absorption. *Eur J Pharm Biopharm* 97, 192-203. doi:10.1016/j.ejpb.2015.09.009
- Palermo, J.-S., Silverstone, A., Riedesel, E. A. et al. (2020). A pilot study on the effect of fat loading on the gastrointestinal tract of healthy dogs. *J Small Anim Pract* 61, 732-737. doi:10.1111/jsap.13216
- Panasevich, M. R., Rossoni Serao, M. C., de Godoy, M. R. C. et al. (2013). Potato fiber as a dietary fiber source in dog foods. *J Anim Sci* 91, 5344-5352. doi:10.2527/jas.2013-6842
- Panasevich, M. R., Kerr, K. R., Serao, M. C. R. et al. (2015). Evaluation of soluble corn fiber on chemical composition and nitrogen-corrected true metabolizable energy and its effects on in vitro fermentation and in vivo responses in dogs. *J Anim Sci* 93, 2191-2200. doi:10.2527/jas.2014-8425
- Park, H.-J., Lee, S.-E., Kim, H.-B. et al. (2015). Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. *J Vet Intern Med* 29, 43-50. doi:10.1111/jvim.12455
- Payne, A. N., Zihler, A., Chassard, C. et al. (2012). Advances and perspectives in in vitro human gut fermentation modeling. *Trends Biotechnol* 30, 17-25. doi:10.1016/j.tibtech.2011.06.011



- Penazzi, L., Schiavone, A., Russo, N. et al. (2021). In vivo and in vitro digestibility of an extruded complete dog food containing black soldier fly (*Hermetia illucens*) larvae meal as protein source. *Front Vet Sci* 8, 653411. doi:10.3389/fvets.2021.653411
- Pereira, G. Q., Gomes, L. A., Santos, I. S. et al. (2018). Fecal microbiota transplantation in puppies with canine parvovirus infection. *J Vet Intern Med* 32, 707-711. doi:10.1111/jvim.15072
- Phillips-Donaldson, D. (2019). Global pet food sales hit \$91 billion in 2018. *Petfoodindustry.com*. <https://www.petfoodindustry.com/articles/7899-global-pet-food-sales-hit-91-billion-in-2018>
- Pilla, R. and Suchodolski, J. S. (2020). The role of the canine gut microbiome and metabolome in health and gastrointestinal disease. *Front Vet Sci* 6, 498. doi:10.3389/fvets.2019.00498
- Propst, E. L., Flickinger, E. A., Bauer, L. L. et al. (2003). A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. *J Anim Sci* 81, 3057-3066. doi:10.2527/2003.81123057x
- Raffan, E., Dennis, R. J., O'Donovan, C. J. et al. (2016). A deletion in the canine POMC gene is associated with weight and appetite in obesity-prone labrador retriever dogs. *Cell Metab* 23, 893-900. doi:10.1016/j.cmet.2016.04.012
- Rahat-Rozenbloom, S., Fernandes, J., Gloor, G. B. et al. (2014). Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans. *Int J Obes* 38, 1525-1531. doi:10.1038/ijo.2014.46
- Rana, S. V., Sharma, S., Malik, A. et al. (2013). Small intestinal bacterial overgrowth and orocecal transit time in patients of inflammatory bowel disease. *Dig Dis Sci* 58, 2594-2598. doi:10.1007/s10620-013-2694-x
- Ricci, R., Perazzi, A., Badon, T. et al. (2018). Effect of storage on long-term stability of salivary α -amylase, lysozyme, lactate dehydrogenase, calcium and phosphorus in dogs. *Vet J* 242, 44-47. doi:10.1016/j.tvjl.2018.10.006
- Ridlon, J. M., Kang, D. J., Hylemon, P. B. et al. (2014). Bile acids and the gut microbiome. *Curr Opin Gastroenterol* 30, 332-338. doi:10.1097/MOG.0000000000000057
- Robin, A. (2007). *Les enteropathies excudatives chez le chien : Actualités, diagnostiques et analyse retrospective de series de cas cliniques*. Lyon, France: Université Claude Bernard.
- Roussel, C., Cordonnier, C., Galia, W. et al. (2016). Increased EHEC survival and virulence gene expression indicate an enhanced pathogenicity upon simulated pediatric gastrointestinal conditions. *Pediatr Res* 80, 734-743. doi:10.1038/pr.2016.144
- Roussel, C., De Paepe, K., Galia, W. et al. (2020). Spatial and temporal modulation of enterotoxigenic *E. coli* H10407 pathogenesis and interplay with microbiota in human gut models. *BMC Biol* 18, 141. doi:10.1186/s12915-020-00860-x
- Russel, W. M. S. and Burch, R. L. (1959). *The Principles of Humane Experimental Technique*. <https://caat.jhsph.edu/principles/the-principles-of-humane-experimental-technique>
- Sagawa, K., Li, F., Liese, R. et al. (2009). Fed and fasted gastric pH and gastric residence time in conscious beagle dogs. *J Pharm Sci* 98, 2494-2500. doi:10.1002/jps.21602
- Salas-Mani, A., Jeusette, I., Castillo, I. et al. (2018). Fecal microbiota composition changes after a BW loss diet in Beagle dogs. *J Anim Sci* 96, 3102-3111. doi:10.1093/jas/sky193
- Sams, L., Paume, J., Giallo, J. et al. (2016). Relevant pH and lipase for in vitro models of gastric digestion. *Food Funct* 7, 30-45. doi:10.1039/C5FO00930H
- Scarsella, E., Stefanon, B., Cintio, M. et al. (2020). Learning machine approach reveals microbial signatures of diet and sex in dog. *PLoS One* 15, e0237874. doi:10.1371/journal.pone.0237874
- Schmidt, M., Unterer, S., Suchodolski, J. S. et al. (2018). The fecal microbiome and metabolome differs between dogs fed bones and raw food (BARF) diets and dogs fed commercial diets. *PLoS One* 13, e0201279. doi:10.1371/journal.pone.0201279
- Schwizer, W., Steingötter, A., Fox, M. et al. (2002). Non-invasive measurement of gastric accommodation in humans. *Gut* 51, Suppl 1, i59-62. doi:10.1136/gut.51.suppl_1.i59
- Shinchi, H., Takao, S., Fukura, K. et al. (1996). Gastric acid secretion and gastroduodenal pH after pancreaticogastrostomy in dogs. *J Surg Res* 61, 152-158. doi:10.1006/jsre.1996.0097
- Simon, M. (2019). *L'anxiété chez le chien, les répercussions sur le microbiote intestinal: Intérêt de l'utilisation des probiotiques dans la prise en charge thérapeutique*. Toulouse, France: Ecole Nationale Vétérinaire de Toulouse. <https://oatao.univ-toulouse.fr/25833/>
- Simpson, K. W., Dogan, B., Rishniw, M. et al. (2006). Adherent and invasive *Escherichia coli* is associated with granulomatous colitis in boxer dogs. *Infect Immun* 74, 4778-4792. doi:10.1128/IAI.00067-06
- Sjögren, E., Abrahamsson, B., Augustijns, P. et al. (2014). In vivo methods for drug absorption – Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. *Eur J Pharm Sci* 57, 99-151. doi:10.1016/j.ejps.2014.02.010
- Smeets-Peeters, M., Watson, T., Minekus, M. et al. (1998). A review of the physiology of the canine digestive tract related to the development of in vitro systems. *Nutr Res Rev* 11, 45-69. doi:10.1079/NRR19980005
- Smeets-Peeters, M. J., Minekus, M., Havenaar, R. et al. (1999). Description of a dynamic in vitro model of the dog gastrointestinal tract and an evaluation of various transit times for protein and calcium. *Altern Lab Anim* 27, 935-949. doi:10.1177/026119299902700608
- Smeets-Peeters, M. (2000). *Feeding FIDO: Development, validation and application of a dynamic, in vitro model of the gastrointestinal tract of the dog*. Wageningen University. Dissertation. <https://www.wur.nl/nl/Publicatie-details.htm?publicationId=publication-way-3635343534>
- Smith, H. W. (1965). Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J Pathol* 89, 95-122. doi:10.1002/path.1700890112
- Souliman, S., Blanquet, S., Beyssac, E. et al. (2006). A level A in



- vitro/in vivo correlation in fasted and fed states using different methods: Applied to solid immediate release oral dosage form. *Eur J Pharm Sci* 27, 72-79. doi:10.1016/j.ejps.2005.08.006
- Suchodolski, J. S., Camacho, J. and Steiner, J. M. (2008). Analysis of bacterial diversity in the canine duodenum, jejunum, ileum, and colon by comparative 16S rRNA gene analysis. *FEMS Microbiol Ecol* 66, 567-578. doi:10.1111/j.1574-6941.2008.00521.x
- Suchodolski, J. S., Dowd, S. E., Westermarck, E. et al. (2009). The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. *BMC Microbiol* 9, 210. doi:10.1186/1471-2180-9-210
- Suchodolski, J. S., Xenoulis, P. G., Paddock, C. G. et al. (2010). Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. *Vet Microbiol* 142, 394-400. doi:10.1016/j.vetmic.2009.11.002
- Suchodolski, J. S. (2011). Companion animals symposium: Microbes and gastrointestinal health of dogs and cats. *J Anim Sci* 89, 1520-1530. doi:10.2527/jas.2010-3377
- Suchodolski, J. S., Dowd, S. E., Wilke, V. et al. (2012a). 16S rRNA gene pyrosequencing reveals bacterial dysbiosis in the duodenum of dogs with idiopathic inflammatory bowel disease. *PLoS One* 7, e39333. doi:10.1371/journal.pone.0039333
- Suchodolski, J. S., Markel, M. E., Garcia-Mazcorro, J. F. et al. (2012b). The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. *PLoS One* 7, e51907. doi:10.1371/journal.pone.0051907
- Sunvold, G. D., Fahey, G. C., Merchen, N. R. et al. (1995a). In vitro fermentation of selected fibrous substrates by dog and cat fecal inoculum: Influence of diet composition on substrate organic matter disappearance and short-chain fatty acid production. *J Anim Sci* 73, 1110-1122. doi:10.2527/1995.7341110x
- Sunvold, G. D., Hussein, H. S., Fahey, G. C. et al. (1995b). In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. *J Anim Sci* 73, 3639-3648. doi:10.2527/1995.73123639x
- Sunvold, G. D., Fahey, G. C., Merchen, N. R. et al. (1995c). Dietary fiber for dogs: IV. In vitro fermentation of selected fiber sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. *J Anim Sci* 73, 1099-1109. doi:10.2527/1995.7341099x
- Swanson, K. S., Dowd, S. E., Suchodolski, J. S. et al. (2011). Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *ISME J* 5, 639-649. doi:10.1038/ismej.2010.162
- Tanner, S. A., Zihler Berner, A., Rigozzi, E. et al. (2014). In vitro continuous fermentation model (PolyFermS) of the swine proximal colon for simultaneous testing on the same gut microbiota. *PLoS One* 9, e94123. doi:10.1371/journal.pone.0094123
- Thakare, R., Alamoudi, J. A., Gautam, N. et al. (2018a). Species differences in bile acids I. Plasma and urine bile acid composition. *J Appl Toxicol* 38, 1323-1335. doi:10.1002/jat.3644
- Thakare, R., Alamoudi, J. A., Gautam, N. et al. (2018b). Species differences in bile acids II. Bile acid metabolism. *J Appl Toxicol* 38, 1336-1352. doi:10.1002/jat.3645
- Theodorakis, M. C. (1980). External scintigraphy in measuring rate of gastric emptying in beagles. *Am J Physiol* 239, G39-43. doi:10.1152/ajpgi.1980.239.1.G39
- Thuenemann, E. C., Mandalari, G., Rich, G. T. et al. (2015). Dynamic gastric model (DGM). In K. Verhoeckx, P. Cotter, I. López-Expósito et al. (eds.), *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*. Cham, Switzerland: Springer.
- Tompkins, T. A., Mainville, I. and Arcand, Y. (2011). The impact of meals on a probiotic during transit through a model of the human upper gastrointestinal tract. *Benef Microbes* 2, 295-303. doi:10.3920/BM2011.0022
- Traughber, Z. T., He, F., Hoke, J. M. et al. (2020). Chemical composition and in vitro fermentation characteristics of legumes using canine fecal inoculum. *Transl Anim Sci* 4, txaa200. doi:10.1093/tas/txaa200
- Tvarijonaviciute, A., Ceron, J. J., Holden, S. L. et al. (2012). Obesity-related metabolic dysfunction in dogs: A comparison with human metabolic syndrome. *BMC Vet Res* 8, 147. doi:10.1186/1746-6148-8-147
- Tzortzis, G., Goulas, A. K., Baillon, M.-L. A. et al. (2004). In vitro evaluation of the fermentation properties of galactooligosaccharides synthesised by alpha-galactosidase from *Lactobacillus reuteri*. *Appl Microbiol Biotechnol* 64, 106-111. doi:10.1007/s00253-003-1427-z
- Van de Wiele, T., Van den Abbeele, P., Ossieur, W. et al. (2015). The simulator of the human intestinal microbial ecosystem (SHIME®). In K. Verhoeckx, P. Cotter, I. López-Expósito et al. (eds.), *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*. Cham, Switzerland: Springer.
- Van den Abbeele, P., Grootaert, C., Possemiers, S. et al. (2009). In vitro model to study the modulation of the mucin-adhered bacterial community. *Appl Microbiol Biotechnol* 83, 349-359. doi:10.1007/s00253-009-1947-2
- Van den Abbeele, P., Taminiau, B., Pinheiro, I. et al. (2018). Arabinoxyloligosaccharides and inulin impact inter-individual variation on microbial metabolism and composition, which immunomodulates human cells. *J Agric Food Chem* 66, 1121-1130. doi:10.1021/acs.jafc.7b04611
- Van den Abbeele, P., Moens, F., Pignataro, G. et al. (2020a). Yeast-derived formulations are differentially fermented by the canine and feline microbiome as assessed in a novel in vitro colonic fermentation model. *J Agric Food Chem* 68, 13102-13110. doi:10.1021/acs.jafc.9b05085
- Van den Abbeele, P., Duysburgh, C., Rakebrandt, M. et al. (2020b). Dried yeast cell walls high in beta-glucan and mannan-oligosaccharides positively affect microbial composition and activity in the canine gastrointestinal tract in vitro. *J Anim Sci* 98, skaa173. doi:10.1093/jas/skaa173
- van Zelst, M., Hesta, M., Alexander, L. G. et al. (2015). In vitro selenium accessibility in pet foods is affected by diet composition and type. *Br J Nutr* 113, 1888-1894. doi:10.1017/S0007114515001324

- Verdier, C., Denis, S., Gasc, C. et al. (2021). An oral FMT capsule as efficient as an enema for microbiota reconstruction following disruption by antibiotics, as assessed in an in vitro human gut model. *Microorganisms* 9, 358. doi:10.3390/microorganisms9020358
- Verstrepen, L., Van den Abbeele, P., Pignataro, G. et al. (2021). Inclusion of small intestinal absorption and simulated mucosal surfaces further improve the mucosal simulator of the canine intestinal microbial ecosystem (M-SCIME™). *Res Vet Sci* 140, 100-108. doi:10.1016/j.rvsc.2021.08.011
- Vierbaum, L., Eisenhauer, L., Vahjen, W. et al. (2019). In vitro evaluation of the effects of *Yucca schidigera* and inulin on the fermentation potential of the faecal microbiota of dogs fed diets with low or high protein concentrations. *Arch Anim Nutr* 73, 399-413. doi:10.1080/1745039X.2019.1616498
- Wang, W.-X., Yan, G.-Z., Sun, F. et al. (2005). A non-invasive method for gastrointestinal parameter monitoring. *World J Gastroenterol* 11, 521-524. doi:10.3748/wjg.v11.i4.521
- Warrit, K., Boscan, P., Ferguson, L. E. et al. (2017). Minimally invasive wireless motility capsule to study canine gastrointestinal motility and pH. *Vet J* 227, 36-41. doi:10.1016/j.tvjl.2017.08.005
- Washizu, T., Ishida, T., Washizu, M. et al. (1994). Changes in bile acid composition of serum and gallbladder bile in bile duct ligated dogs. *J Vet Med Sci* 56, 299-303. doi:10.1292/jvms.56.299
- Weber, M. (2006). Influence of size on the dog's digestive function. *Bul de l'Ac Vét de France*, 327. doi:10.4267/2042/47851
- Weber, M. P., Biourge, V. C. and Nguyen, P. G. (2017). Digestive sensitivity varies according to size of dogs: A review. *J Anim Physiol Anim Nutr (Berl)* 101, 1-9. doi:10.1111/jpn.12507
- Williams, B. A., Bosch, M. W., Boer, H. et al. (2005). An in vitro batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Anim Feed Sci Technol* 123-124, 445-462. doi:10.1016/j.anifeedsci.2005.04.031
- Xenoulis, P. G., Palculict, B., Allenspach, K. et al. (2008). Molecular-phylogenetic characterization of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. *FEMS Microbiol Ecol* 66, 579-589. doi:10.1111/j.1574-6941.2008.00556.x
- Xu, J., Verbrugghe, A., Lourenço, M. et al. (2016). Does canine inflammatory bowel disease influence gut microbial profile and host metabolism? *BMC Vet Res* 12, 114. doi:10.1186/s12917-016-0736-2
- Xu, J., Verbrugghe, A., Lourenço, M. et al. (2017). The response of canine faecal microbiota to increased dietary protein is influenced by body condition. *BMC Vet Res* 13, 374. doi:10.1186/s12917-017-1276-0
- You, I. and Kim, M. J. (2021). Comparison of gut microbiota of 96 healthy dogs by individual traits: Breed, age, and body condition score. *Animals* 11, 2432. doi:10.3390/ani11082432
- Zeng, R., Zhang, Y. and Du, P. (2014). The SNPs of melanocortin 4 receptor (MC4R) associated with body weight in beagle dogs. *Exp Anim* 63, 73-78. doi:10.1538/expanim.63.73
- Zentek, J. and Meyer, H. (1995). Normal handling of diets – Are all dogs created equal? *J Small Anim Pract* 36, 354-359. doi:10.1111/j.1748-5827.1995.tb02949.x
- Zhang, Y., Jeon, M., Rich, L. J. et al. (2014). Non-invasive multimodal functional imaging of the intestine with frozen micellar naphthalocyanines. *Nat Nanotechnol* 9, 631-638. doi:10.1038/nnano.2014.130

Conflict of interest

The authors have no conflict of interest.