ALTERNATIVES TO ANIMAL EXPERIMENTATION

Intestinal simulators in the 3R context

7 June 2023

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CHALLENGES FOR NUTRITIONAL STUDIES IN PET FOOD INDUSTRY

3R context

- *Nutritional studies include **multiple processes** like physicochemical , mechanical and microbial parameters*
- ***Mode of action** often not possible or challenging to study in vivo*
- *In vivo nutrition & health studies around pets might **feel contradictory** for pet owners*
- *Economical, ethical and societal aspects*

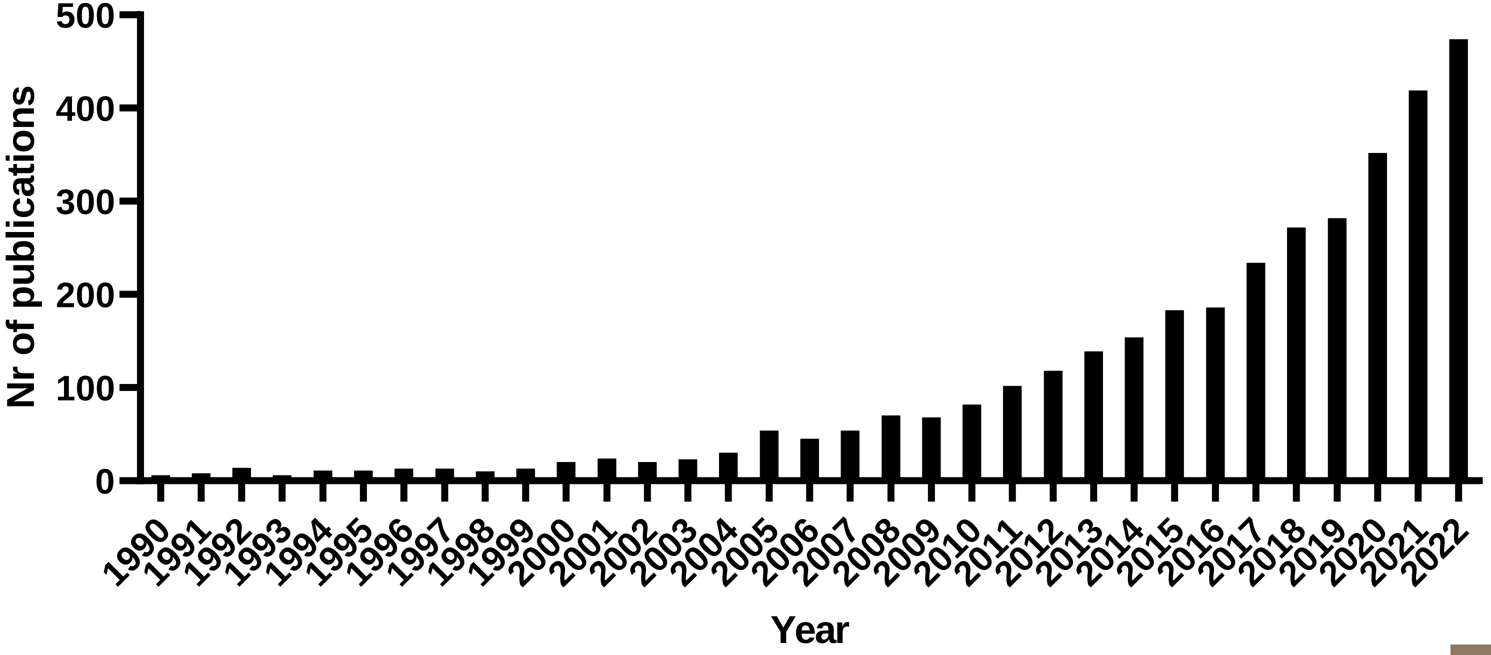
Solution: In vitro intestinal models to reduce and refine and in some cases replace animal experimentation

DEVELOPMENT OF INTESTINAL SIMULATORS

Origin around 1990s: all for human

Static simulations
Upper digestion (INFOGEST)
Batch fermentation

(semi) Dynamic simulations
TIM1: Upper digestion
SHIME: Full digestion



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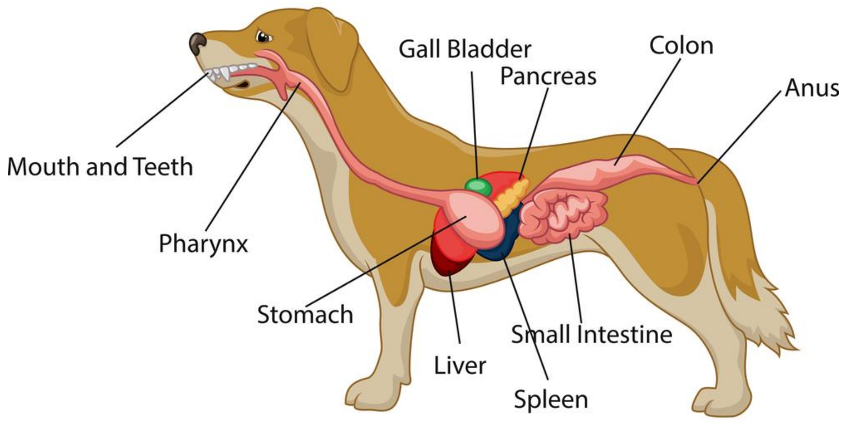
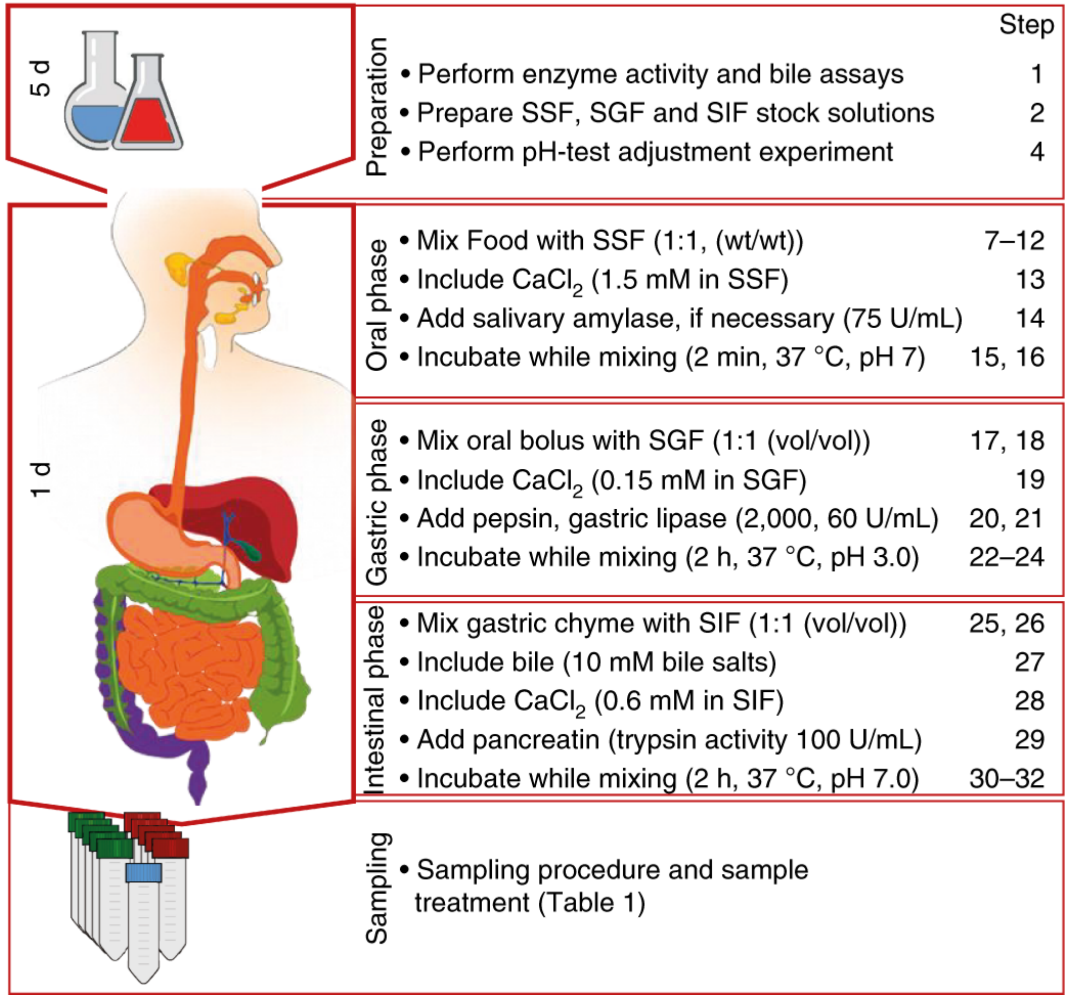
INTESTINAL SIMULATOR TECHNOLOGY

Upper GI tract

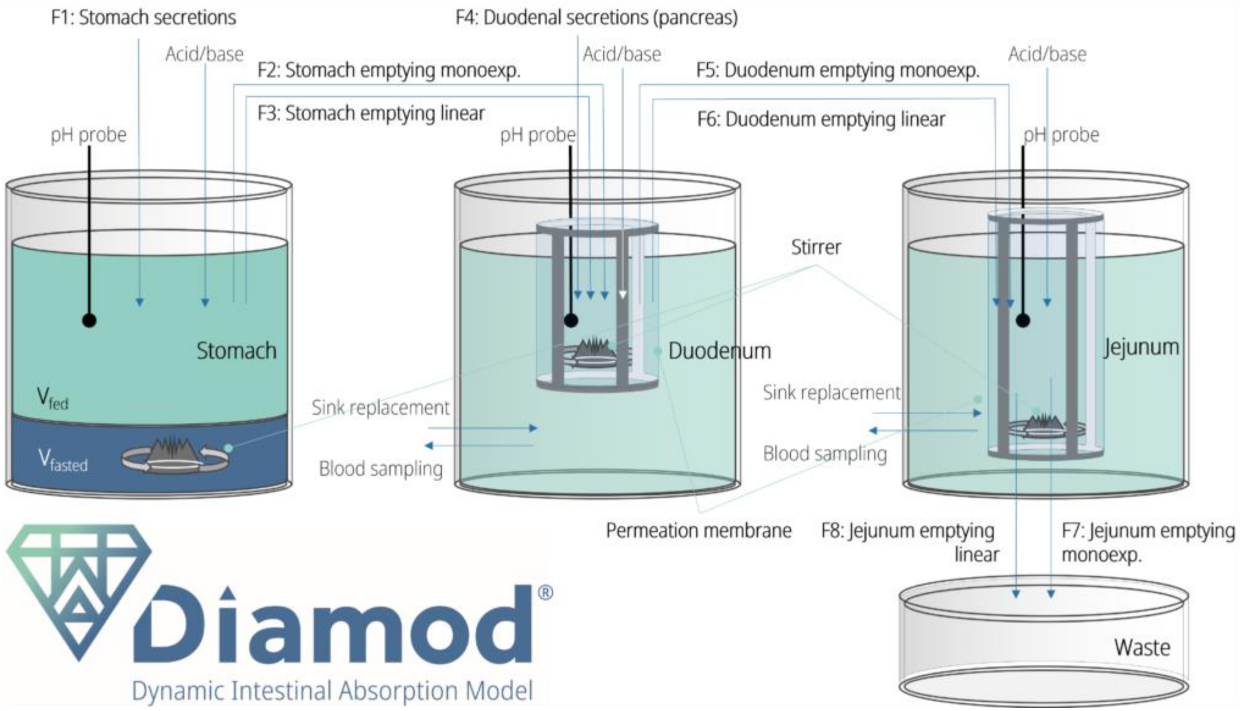
Static digestion models (Cost-Infogest consensus method) → Human
Semi-static digestion models (ProDigest with absorption membrane) → Human
Dynamic digestion model TIM1 (Tim-Company) → Human, pig, dog



UPPER GIT SIMULATIONS



Static INFOGEST consensus method

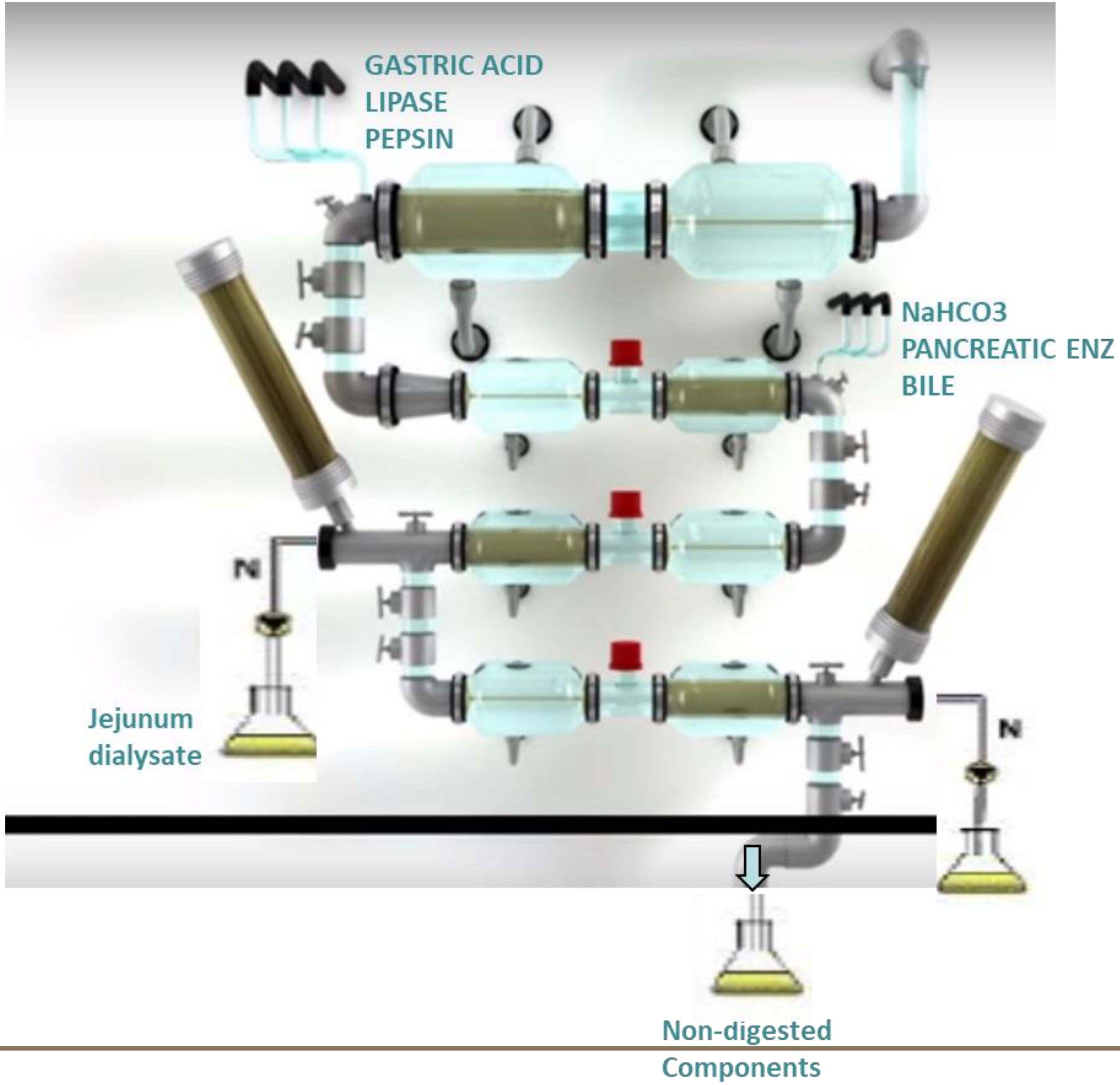


Semi- dynamic with dialysis ProDigest (BE)

UPPER GIT SIMULATIONS

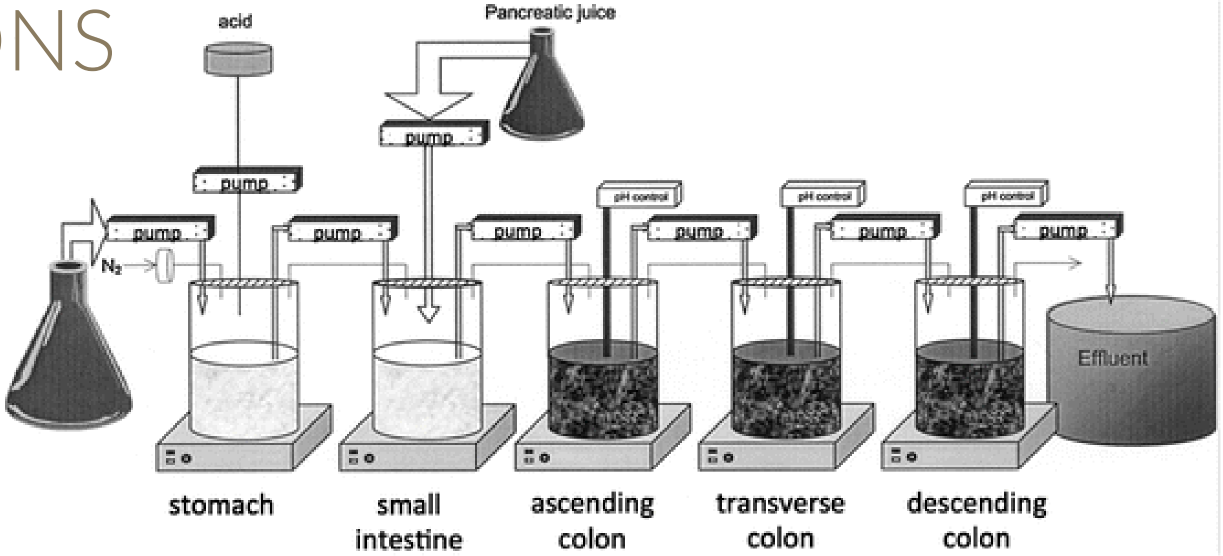
TIM1
TIM-Company (NL)

- Fully dynamic
- Bio-accessibility
- Probiotic survival

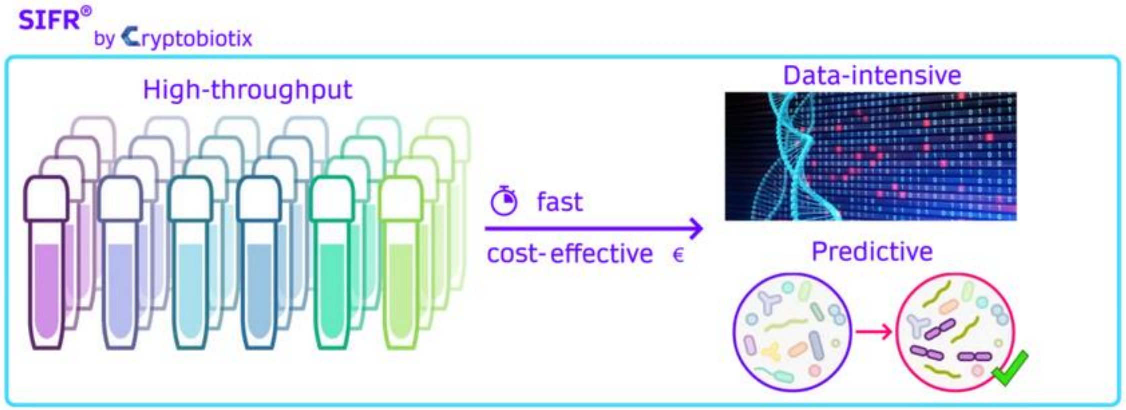


LOWER/FULL GIT SIMULATIONS

TIM2 colonic fermentation
Can be linked to TIM1
(TIM-Company)

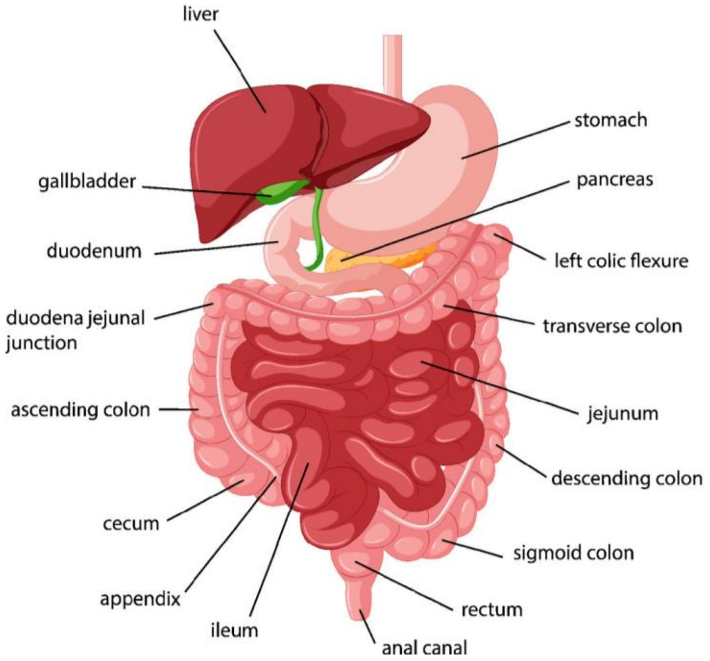


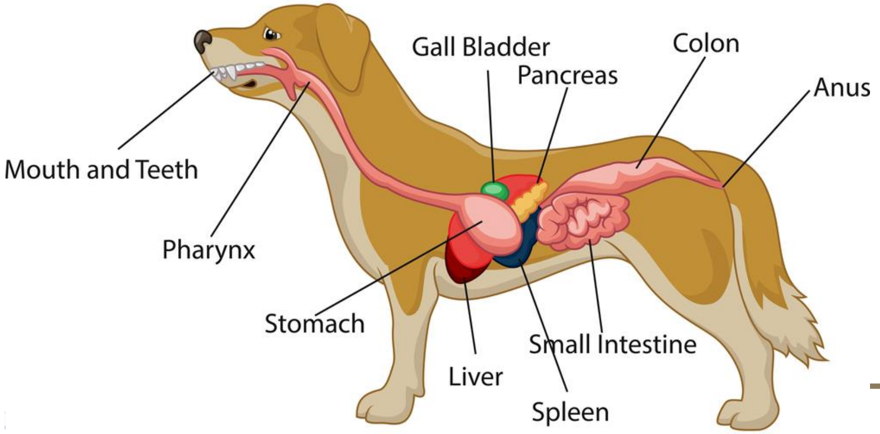
SHIME: dynamic full gut simulator (ProDigest)

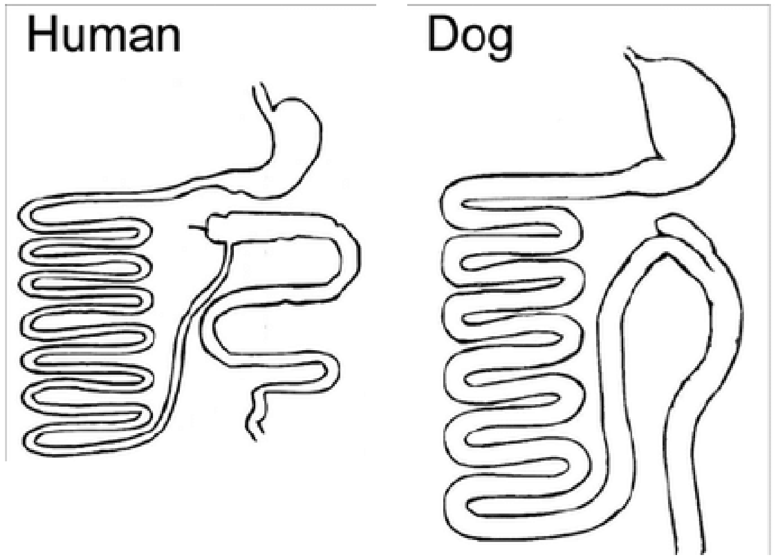


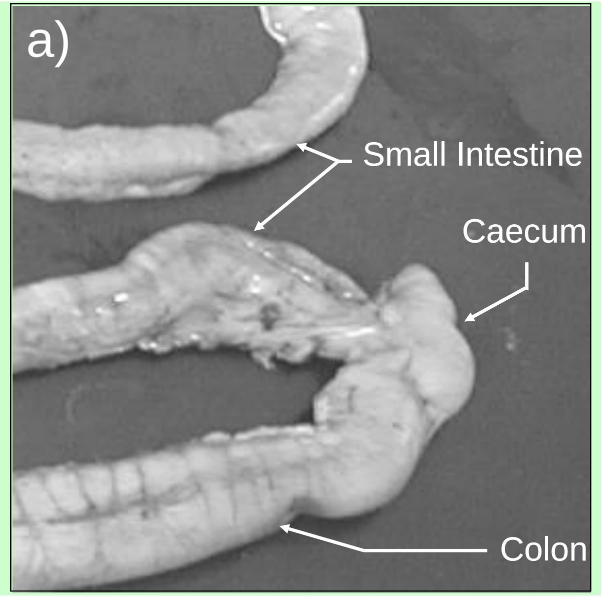
SIFR: High-throughput fermentation
(Cryptobiotix)

HUMAN VS DOG









Main differences

- Length
- Transit time
- pH
- Enzyme concentrations
- Temperature
- Diet
- Microbial community

UPPER GIT

Physiological specificity

Human vs Dog

Entire GIT

- Length
- Complexity

MOUTH

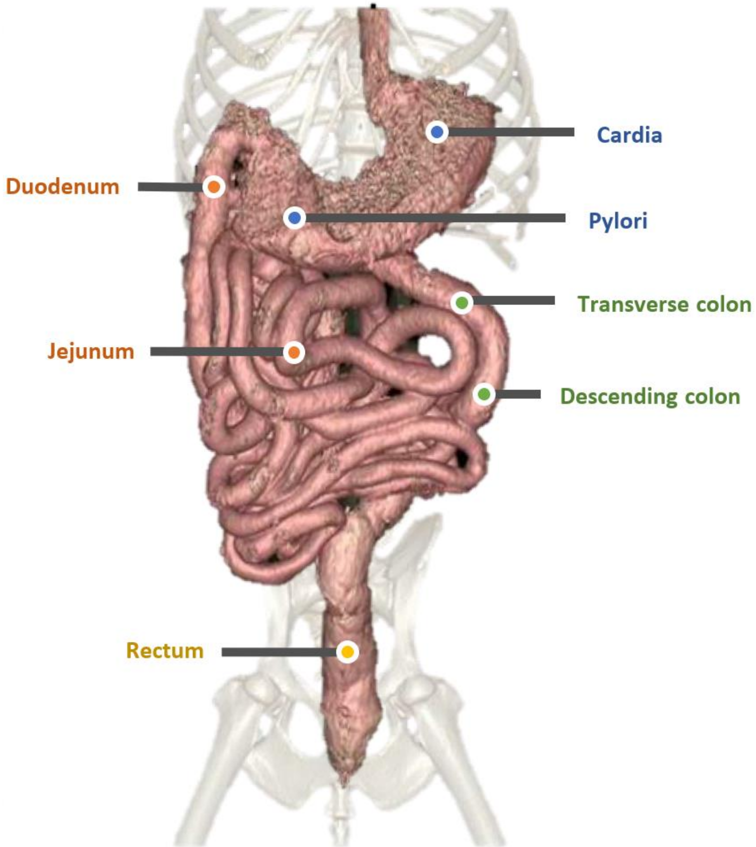
- Mastication
- Enzymes: Amylase
- Salivary flow rate: x 7.5
- Salivary pH: 7.3-7.8

STOMACH

- Gastric pH: 1.7-3.5
- Enzymes: Pepsin, Lipase
- Dilatation capacity: 0.5-8 L
- Mucus: 425-580 µm
- Emptying time: 6.8-15 h

BILE ACIDS

- Gallbladder BA concentration: 40-90 mmol/L
- Total faecal bile acids: 3.4-7.5 µg/mg lyoph. feces
- Faecal bile acids Ratio I/II: 16 % BA I, 85 % BA II

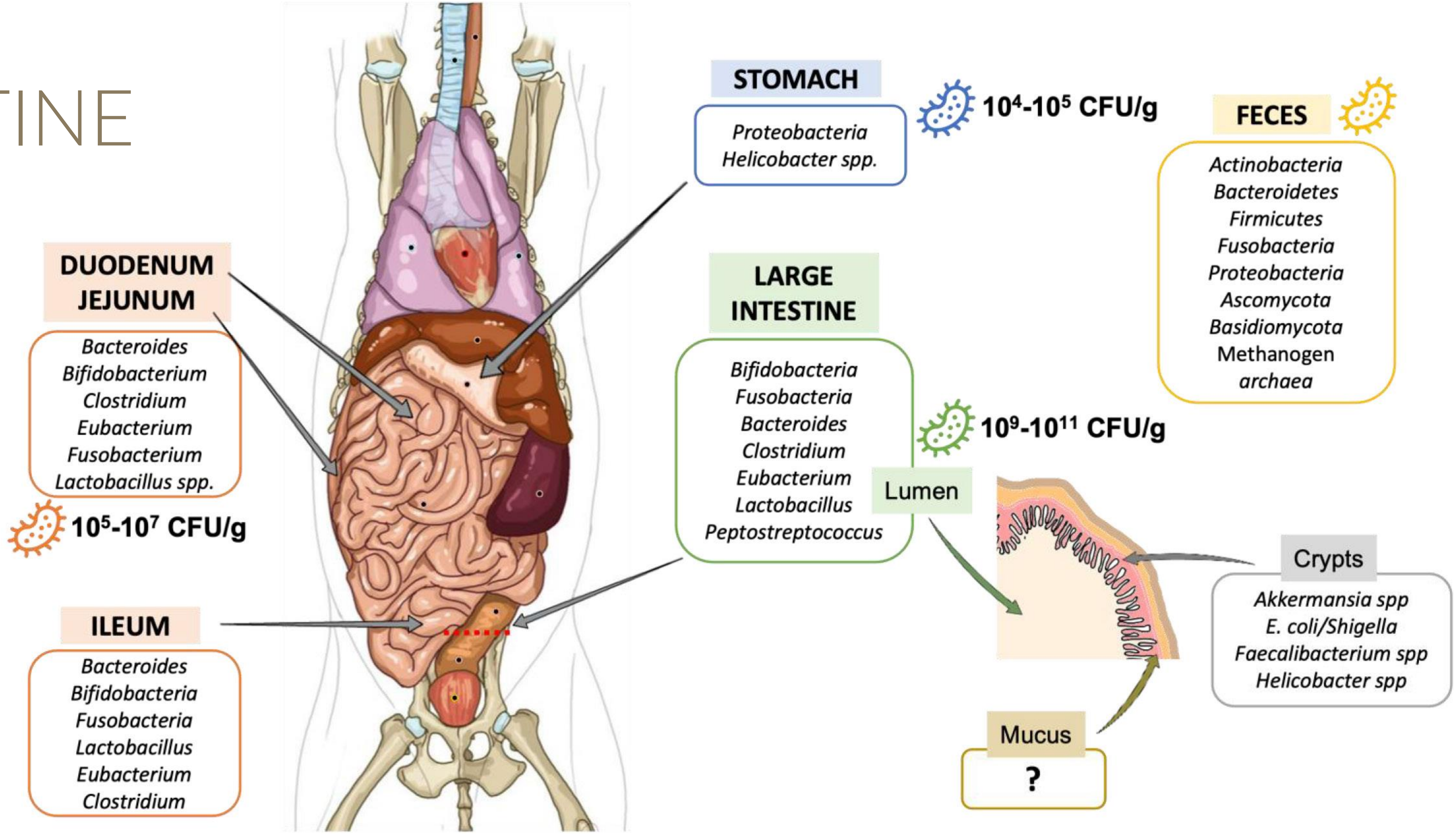


SMALL INTESTINE

- Length: 1-3 m, 5 % ileum
- Intestinal pH: 6.8
- Transit time: 1.9 h
- Motility index: 134 mmHg *s/min

Deschamps et al., 2022

LARGE INTESTINE

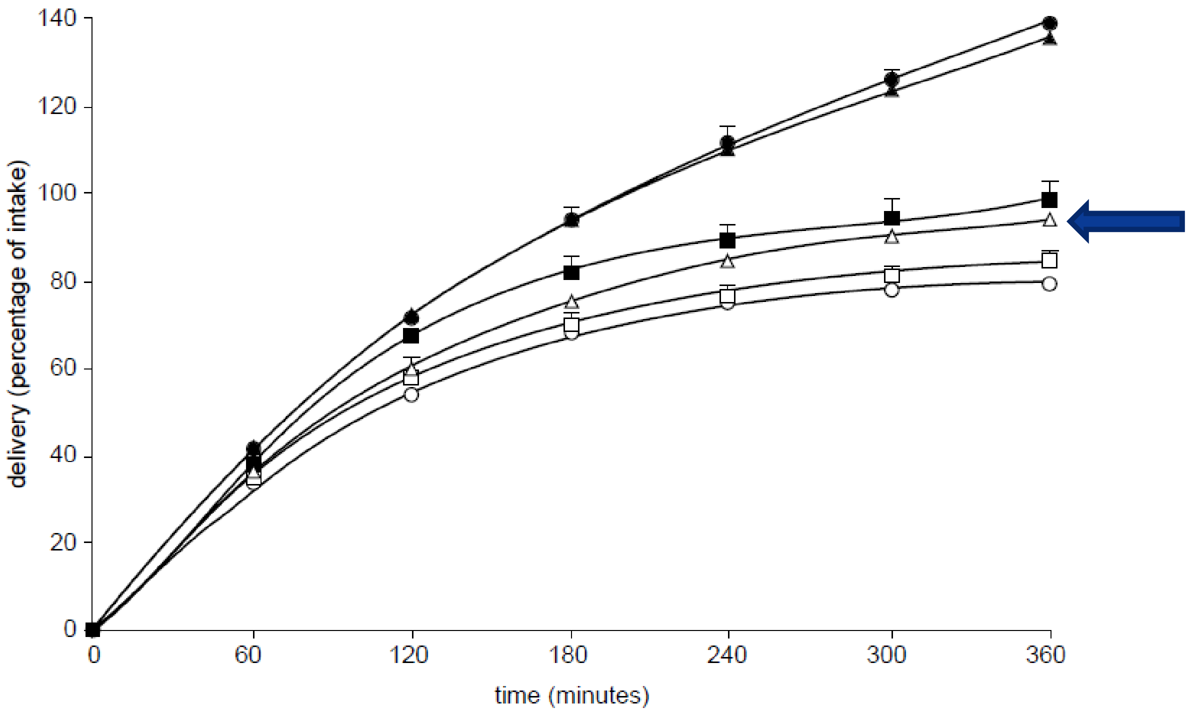


Deschamps et al., 2022

VALIDATION STUDY FIDO TIM1

FIDO: Functional gastroIntestinal DOg model

- Integrates all upper digestive compartments: stomach, duodenum, jejunum and ileum
- Simulates body temperature, gastric and intestinal pH kinetics, gastric and ileal emptying curve, transit time, mixing and delivery of digestive secretions
- Validation comparing gastric meal delivery with different meals vs the average in vivo gastric emptying



● = fresh matter emptying of canned dog food ; ○ = dry matter emptying of canned dog food; ■ = dry matter emptying of dry dog food, particles ≤ 1mm; □ = dry matter emptying of dry dog food, particles ≤ 3mm; ▲ = fresh matter emptying of dry dog food; Δ = preset gastric delivery curve.

Average in vivo gastric emptying curve

Smeets-Peeters et al.,

VALIDATION STUDY FIDO TIM1

Computer programming to mimic the in vivo emptying curves

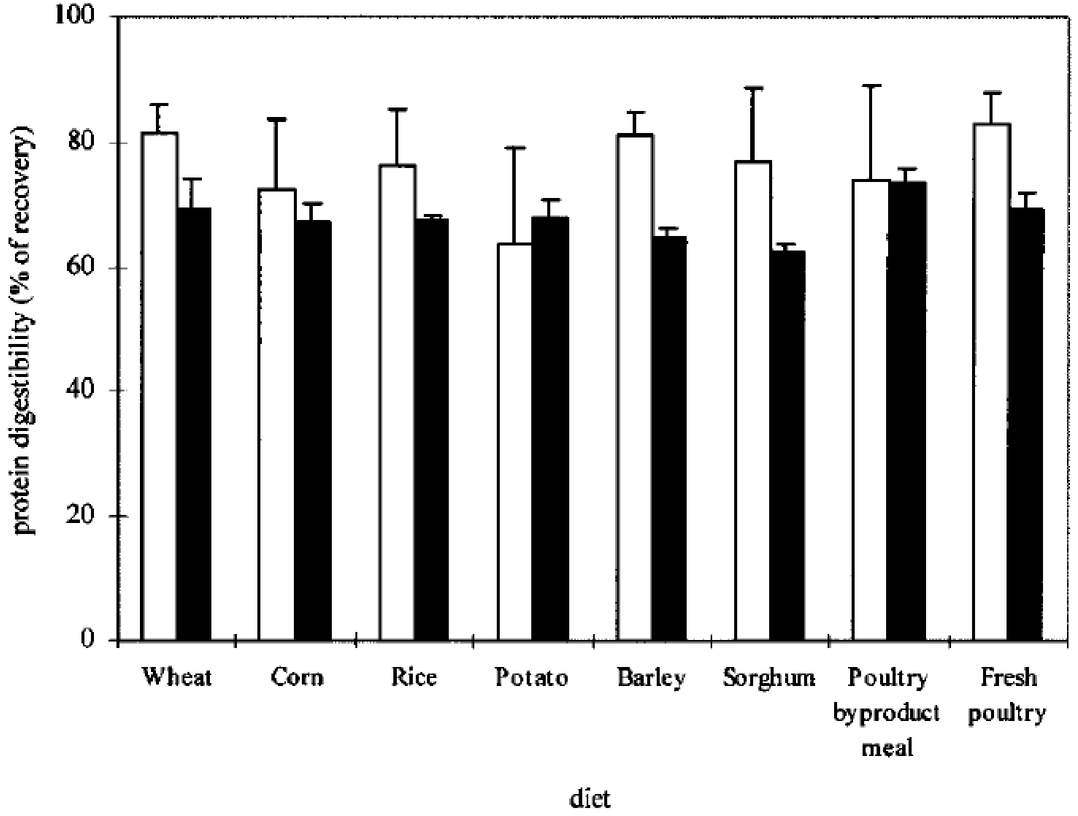


Figure 3 Comparison between the mean (± sd) protein digestibility values for ileally cannulated dogs (white bars; Murray *et al.*, 1997; 1999) and the mean (± range) protein digestibility values (% of input) in the *in vitro* gastrointestinal dog model (black bars) calculated according to Formula 1

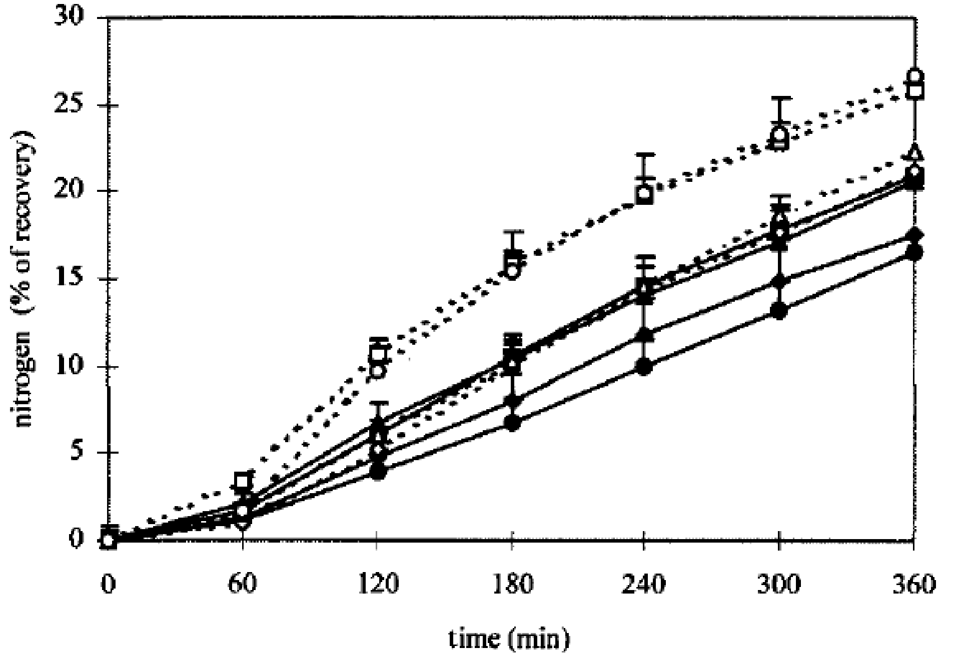


Figure 5 Mean cumulative ileal delivery of N (± range) of 8 different diets varying in starch source or in poultry product component in the *in vitro* canine gastrointestinal model. Solid lines: ◆ wheat, ■ corn, ▲ rice, ● potato and dashed lines:◇ barley, □ sorghum, ▵ poultry by-product meal, ○ fresh poultry

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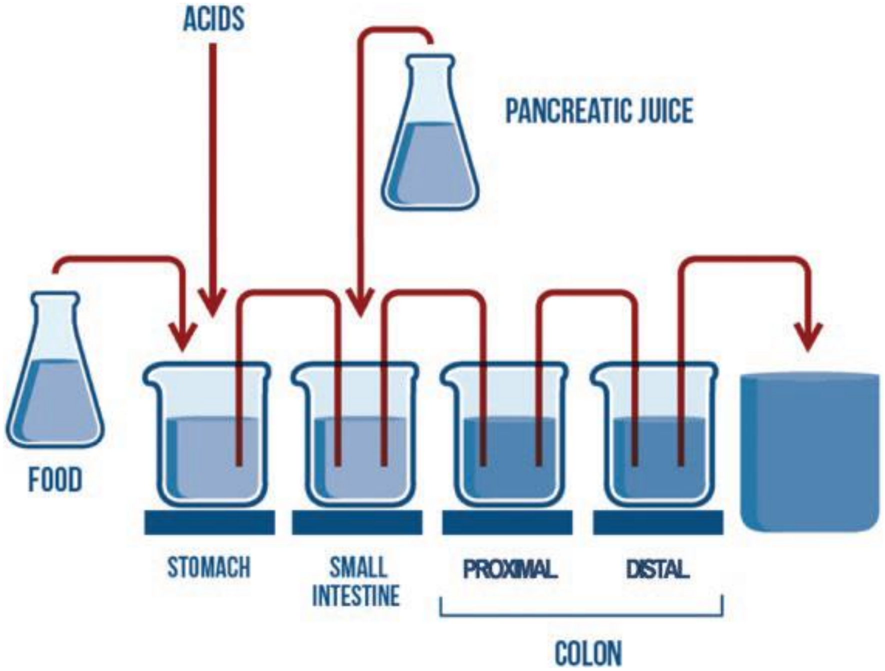
Smeets-Peeters et al., 1998

VALIDATION STUDY SCIME



Duysburgh et al., 2020

VALIDATION STUDY SCIME

	SCIME			SHIME		
	Volume, mL	Residence time, h	pH	Volume, mL	Residence time, h	pH
Stomach	140	1	2.00	200	2	2.00
Small intestine	200	4	6.80	200	4	6.80
Proximal colon	100	6	5.60 to 5.90	500	20	5.60 to 5.90
Transverse colon	—	—	—	800	32	6.10 to 6.40
Distal colon	167	10	6.65 to 6.90	600	24	6.60 to 6.40
Feeding regimen		2×/day			3×/day	

Parameters SHIME vs SCIME

Duysburgh et al., 2020

MICROBIAL COMPARISON

Awareness on bias

→ M-SCIME development with a mimicked mucosal part

	In vitro				In vivo	
	PC		DC			
	%	SEM	%	SEM	%	SEM
Actinobacteria	4.9	1.0	4.9	1.0	3.8	0.6
Bifidobacterium	1.0	0.3	1.0	0.2	0.5	0.1
Coriobacteriaceae	3.8	0.7	4.0	0.7	3.6	0.7
Bacteroidetes	**40.1[b]**	0.8	**28.8[c]**	1.9	1.2[a]	0.2
Bacteroides	**10.5[b]**	0.6	**10.5[b]**	1.8	0.3[a]	0.1
Prevotellaceae	**29.5[b]**	1.3	**18.2[c]**	1.5	0.8[a]	0.2
Uncultured Bacteroidetes	0.1	0.1	0.1	0.0	0.1	0.1
Firmicutes	**52.4[b]**	0.5	**48.6[b]**	0.7	94.4[a]	0.1
Lactobacillus	17.2[a,b]	1.5	**6.3[b]**	0.4	26.7[a]	4.1
Streptococcus	0.1	0.1	<0.1	<0.1	5.0	2.1
Blautia	0.7	0.2	1.0	0.4	1.5	0.3
Clostridium cluster XIVa	0.6	0.1	1.1	0.2	<0.1	<0.1
Clostridium cluster XI	**0.9[b]**	0.1	**1.1[b]**	0.1	17.3[a]	2.4
Uncultured Clostridiales	0.1	0.1	0.8	0.1	0.1	0.3
Allobaculum	7.2	0.6	13.5	0.4	13.3	4.0
Catenibacterium	0.1	<0.1	<0.1	<0.1	2.4	0.7
Erysipelotrichaceae	0.1	<0.1	0.0	0.1	2.3	0.6
Turicibacter	**0.1[b]**	<0.1	**<0.1[b]**	<0.1	14.8[a]	3.3
Uncultured Acidaminococcaceae	0.5	0.1	0.8	0.1	<0.1	<0.1
Megamonas	**19.1[b]**	1.0	**20.5[b]**	1.1	0.1[a]	0.1
Uncultured Firmicutes	5.8[a,b]	0.4	**3.3[b]**	0.3	11.1[a]	1.9
Fusobacteria	0.3[a]	0.1	**14.5[b]**	0.8	0.3[a]	0.1
Uncultured Fusobacteriaceae	0.3[a]	0.1	**14.5[b]**	0.8	0.3	0.1
Proteobacteria	**2.3[b]**	0.2	**3.2[b]**	0.2	<0.1[a]	<0.1
Sutterella	**1.2[b]**	0.1	**1.8[b]**	0.2	<0.1[a]	<0.1
Anaerobiospirillum	**1.0[b]**	0.2	0.3[a,b]	0.1	<0.1[a]	<0.1
Pseudomonas	0.1[a]	0.1	**1.1[b]**	0.2	<0.1[a]	<0.1

Duysburgh et al., 2020

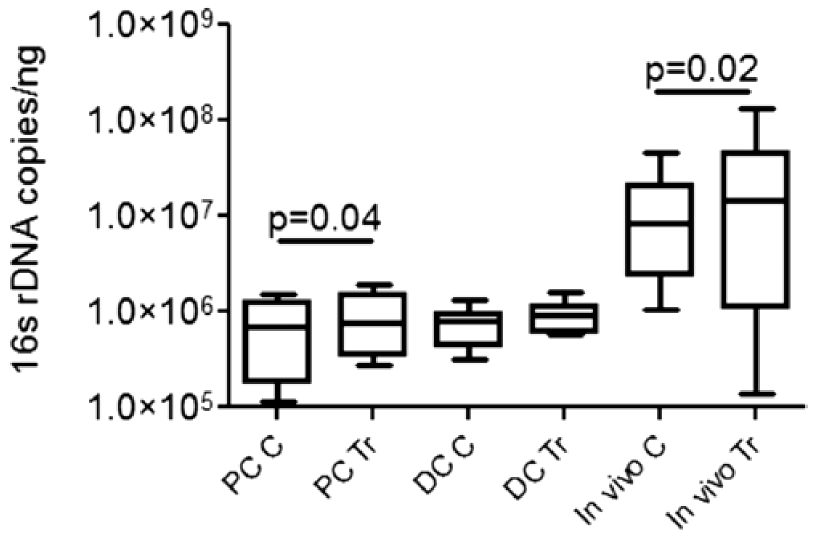
TEST STUDY: FRUCTO OLIGOSACCHARIDES TREATMENT

Metabolic activity: SCFAs, BCFAs, ammonium
Microbial composition

Metabolic activity FOS treatment

	In vitro PC			In vitro DC			In vivo		
	C	TR	P-value	C	TR	P-value	C	TR	P-value
Acetate (mM)	39 ± 4	45 ± 5	0.018	39 ± 3	43 ± 4	0.040	55 ± 7	49 ± 12	0.066
Propionate (mM)	39 ± 3	60 ± 5	<0.001	36 ± 3	46 ± 3	<0.001	42 ± 7	41 ±10	0.526
Butyrate (mM)	14 ± 2	18 ± 4	0.011	19 ± 2	28 ± 2	<0.001	22 ± 9	15 ± 8	0.003
Branched SCFA (mM)	2.7 ± 0.1	2.1 ± 0.1	<0.001	3.1 ± 0.2	2.7 ± 0.1	<0.001	1.0 ± 0.3	0.5 ± 0.3	<0.001
Ammonium (mg/L)	427 ± 13	358 ± 9	<0.001	527 ± 17	449 ± 12	<0.001	292 ± 61	222 ± 63	0.002

UGent Veterinary school
Beagle study



Copies of lactobacillus

Duysburgh et al., 2020

Perspectives

OPEN INNOVATION COLLABORATION

FOLLOW THE EXAMPLE OF HUMAN DIGESTION STUDIES WITH INFOGEST CONSENSUS METHOD

CHALLENGES

- Harmonizing methods
- Case specific models
- Regulation in vitro work
- Very limited data on cat available

Support petfood applications:
- Data support for new nutritional solutions
- Data around mode of action (existing and new nutritional solutions)
- Data on probiotic ability to restore microbial dysbiosis
- Host-microbe interactions

Support pharma:
- Assessment of new microbial restoration strategies (FMT)
- Generate data around drug release, absorption, metabolization etc.
- Support validation of new product formulations

DEVELOPMENTS

- Disease states
- Long vs short term
- Different breeds – sizes – age
- Consensus methods

THANK YOU FOR YOUR ATTENTION

Special thanks to:
Elyse Parent
Achraf Adib Lesaux
Nabil Bosco

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