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# Physiological properties, composition and structural profiling of gastrointestinal mucus in preclinical species

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# Acknowledgement



Vetenskapsrådet



European Research Council  
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SciLifeLab





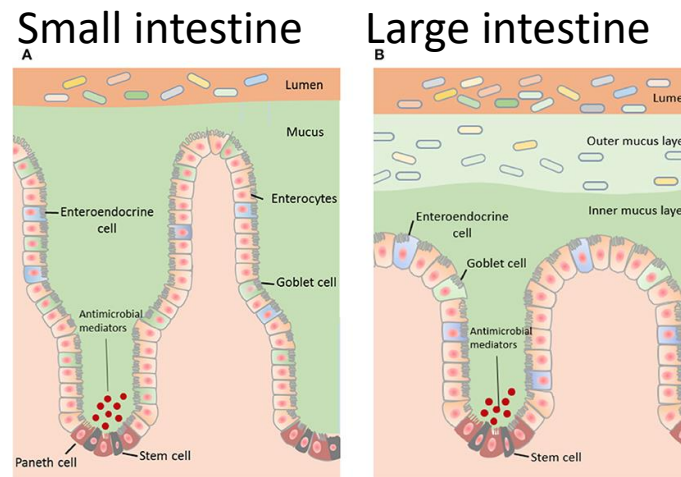
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# GASTROINTESTINAL MUCUS



# Gastrointestinal (GI) mucus

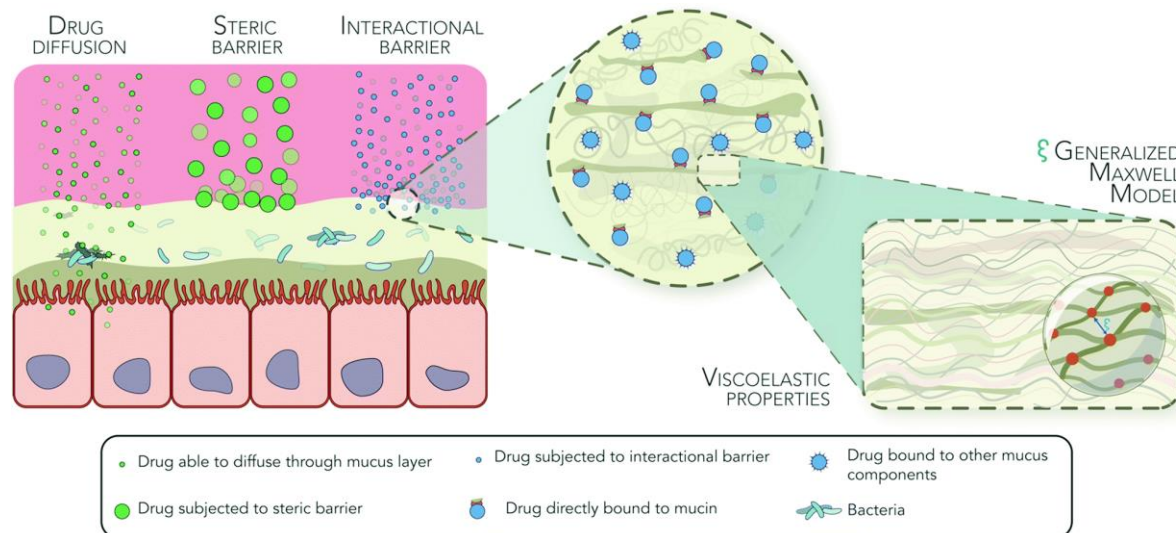
- Hydrogel, covers the gastrointestinal epithelium
- Protects the epithelium
  - Bacteria
  - Noxious substances
- Lubricate luminal contents, facilitating movement





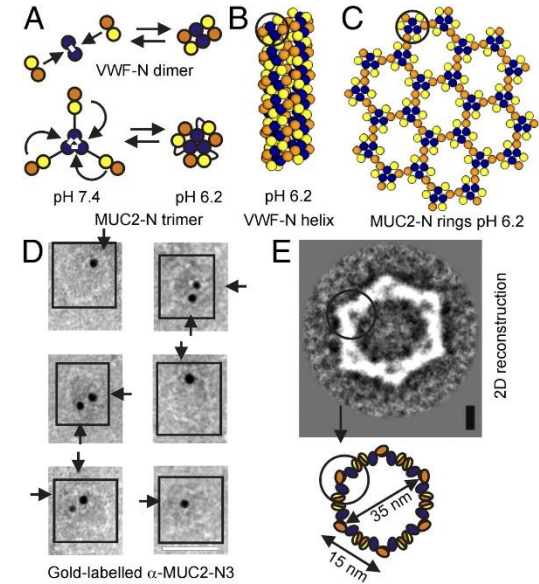
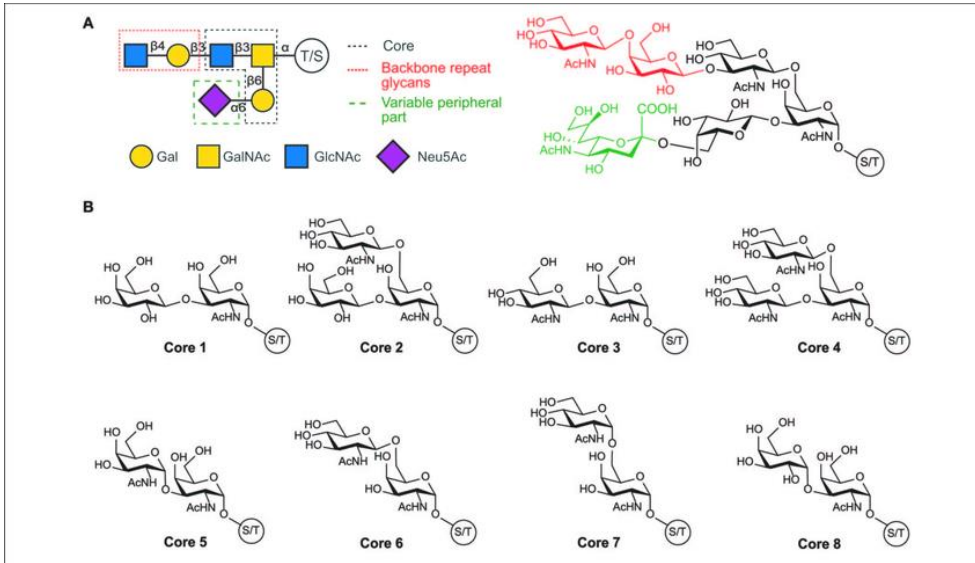
# Role in drug delivery and disposition

- Drugs and drug carriers need to diffuse across this hydrogel
- Challenges
  - Rheology: viscosity and viscoelastic properties
  - Steric hindrance: cross-linked structure, electrostatics
  - Microenvironment: pH, lipids, proteins, DNA etc



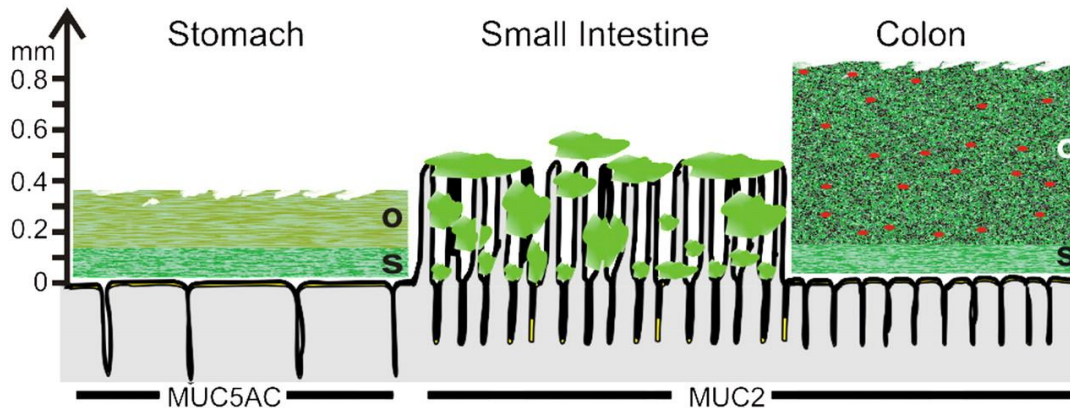


# Mucus organisation



## Mucin backbone: glycans

## Muc-2 structure



O=outer, loose layer  
S = stratified layer  
● = bacteria



## Current knowledge platform

- Studies mainly in rats and mice
- Some studies on mucus from separate GI segments of large animals (mainly focused on porcine jejunal and canine gastric mucus)
- *GI mucus data from large species is lacking*
- *Common and upcoming preclinical larger species: dogs and pigs*
- *Translation of data from preclinical species to human: commonalities and differences*

Atuma et al., Am. J. Physiol. Gastrointest. Liver Physiol., 2001; Phillipson et al., Am. J. Physiol.

Gastrointest. Liver Physiol., 2002; Larhed et al., Pharm. Res., 1998; Bell et al., Gastroenterology, 1985



## On-going studies within Colotan and SweDeliver

- Characterization of mucus in the **full GI tract of pigs and dogs**
- Characterization of **healthy and disease state human colon** with focus on IBD and CRC
- 3R approach – develop artificial colonic mucus
  - Do we need to account for species differences?
- In vitro models for drug and carrier diffusion studies
- Useful parameters for in silico modelling (PBPK/PBB modelling)
- Computational diffusion studies (CFD, MD simulations etc)



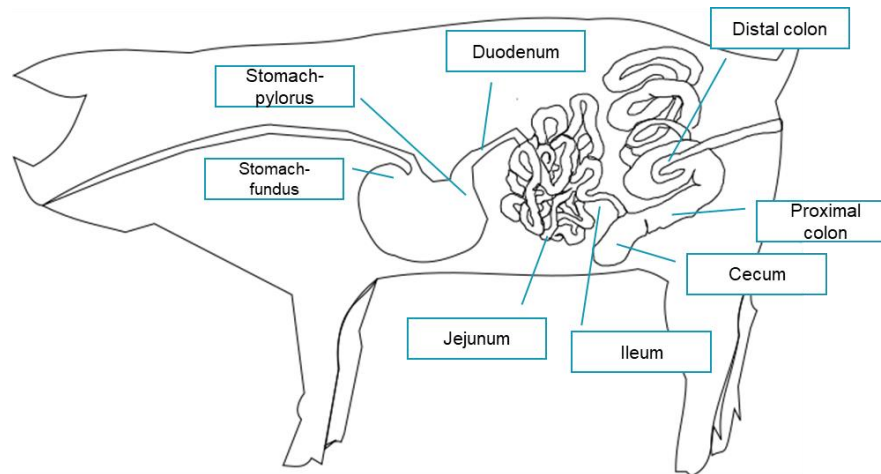


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# PRECLINICAL SPECIES: PIGS & DOGS

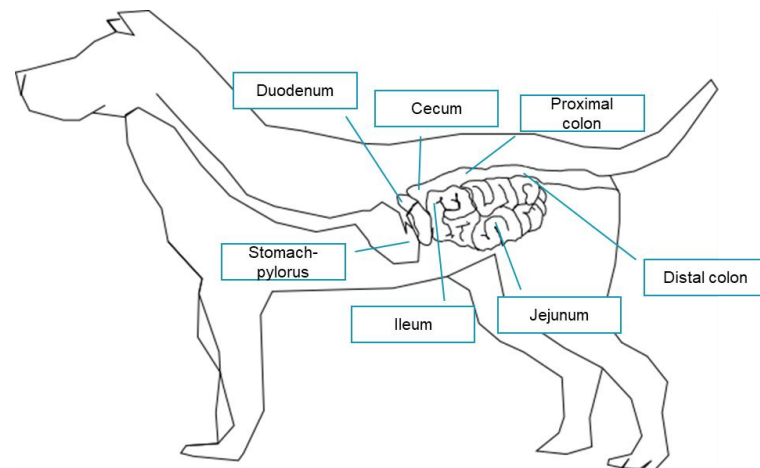
## Pig – a relevant preclinical species in the drug development chain?

- Increasingly used in preclinical assessments
- Suitable for prediction of most ADMET endpoints
- GIT similarities with similar microbiome



# Dog – an established preclinical model in the drug development chain

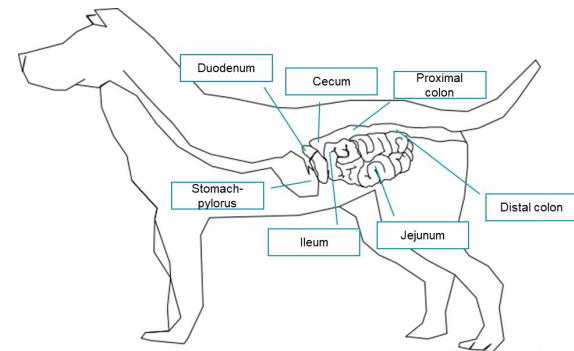
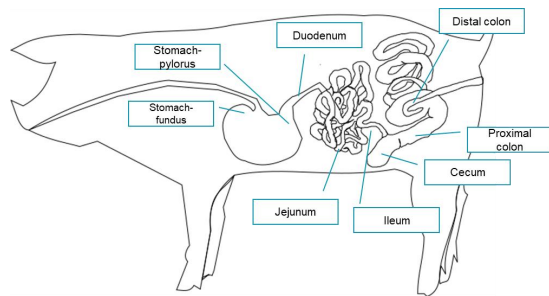
- Commonly used preclinical species for evaluation of controlled release formulations
- GIT similarities, gastric motility similar to humans
- Potential to predict food effects





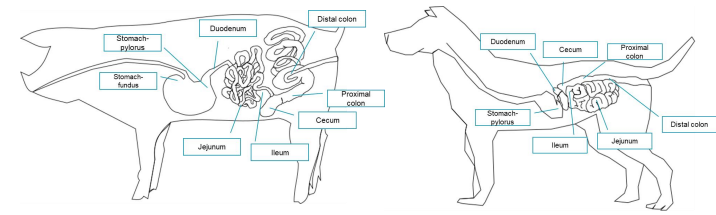
# GI sources for the presented data – animals put down for other reasons than this study

- Crossbreed pigs from a local abattoir <1h from slaughter
- Fasted  $\geq 12$ h prior to slaughter with water ad libitum
- Collection of mucus and intraluminal contents
- Labrador dogs (AZ) and privately owned dog(s) (SLU)
- Tissue from AZ transferred to UU (buffer ~6h)
- Tissue from SLU collected immediately after euthanasia
- Collection of mucus and intraluminal contents

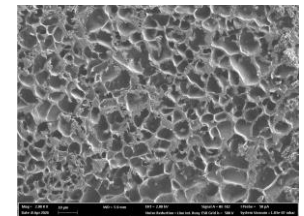
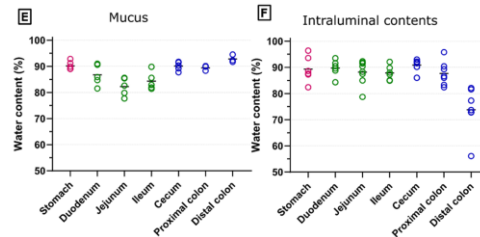
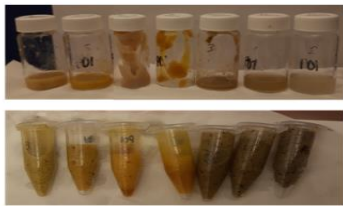




# Characteristics and methods

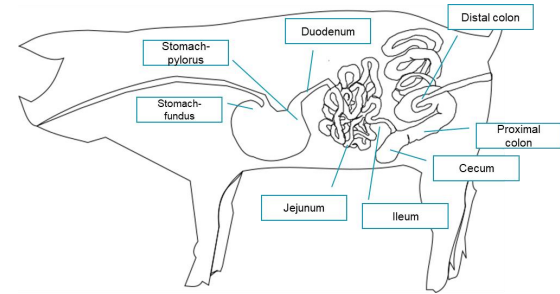


- Color/texture
- pH
- Water content
- Composition
- Viscosity and viscoelastic properties
- Structure
- Visual inspection
- pH (gel, small scale) electrode
- Freeze-drying
- Proteomics<sup>1</sup> (global & targeted), lipidomics & metabolomics<sup>2</sup>
- Rheometer
- Cryo-SEM<sup>3</sup> + ImageJ

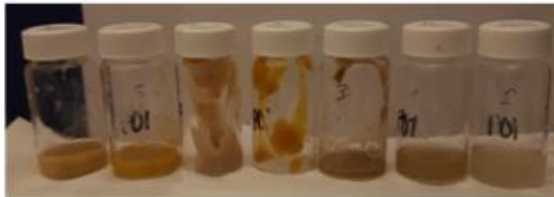


<sup>1</sup>Clinical Proteomics Mass Spectrometry facility, Karolinska Institutet/ Karolinska University Hospital/ Science for Life Laboratory; <sup>2</sup>Swedish Metabolomics Centre (SLU/UmU); <sup>3</sup>Umeå Core Facility for Electron Microscopy (UCEM); Detailed description of methods available in Barmapsalou et al., Eur J Pharm Biopharm, 2021

# Physiological properties



Mucus

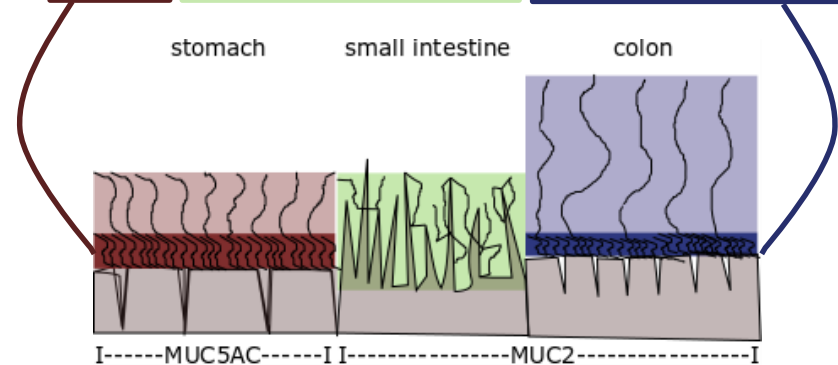


Luminal content

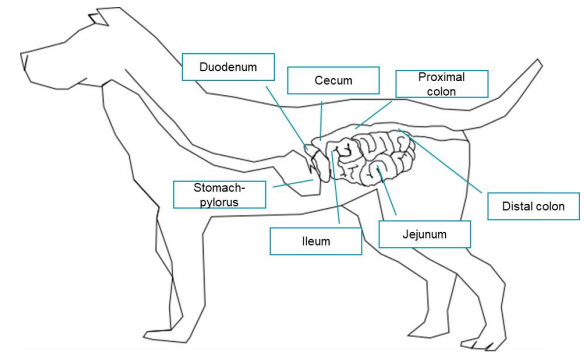


Yield- 'Easiness' of collection

|       | Stomach  | Duodenum  | Jejunum   | Ileum    | Cecum    | Proximal colon | Distal colon |
|-------|----------|-----------|-----------|----------|----------|----------------|--------------|
| Mean  | 3.5%     | 13.4%     | 11.8%     | 5.7%     | 3.1%     | 1.1%           | 1.2%         |
| Range | 1.9-5.2% | 6.2-25.3% | 3.8-19.8% | 2.5-8.9% | 1.3-4.4% | 0.3-1.9%       | 0.5-2.1%     |

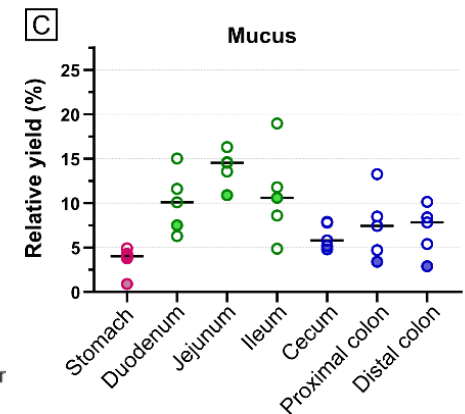
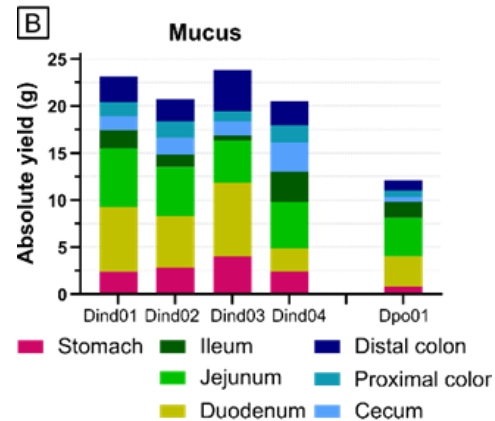
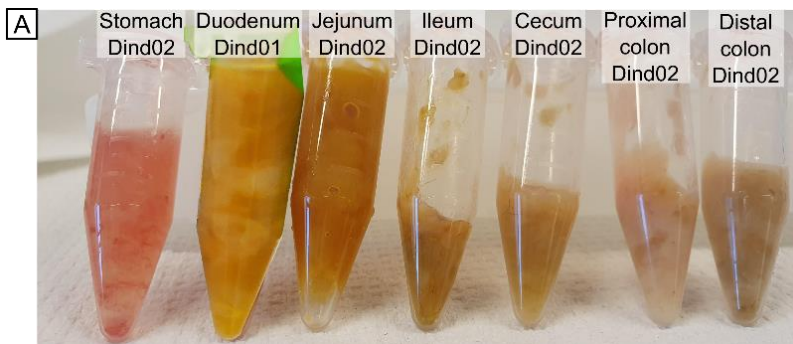


# Physiological properties



## Mucus

## Yield – 'easiness' of collection

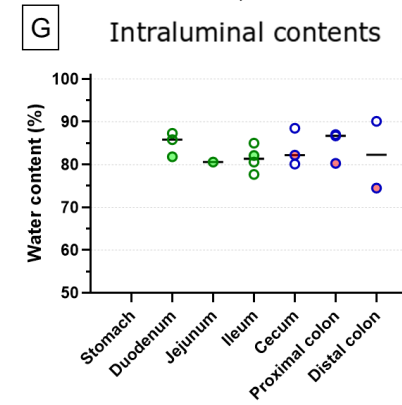
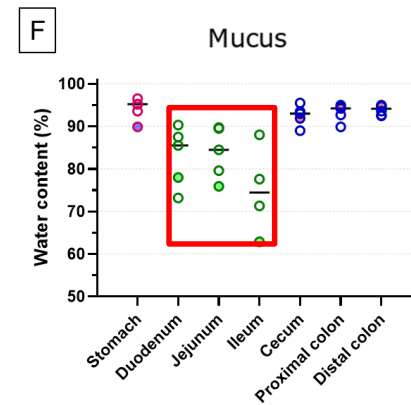
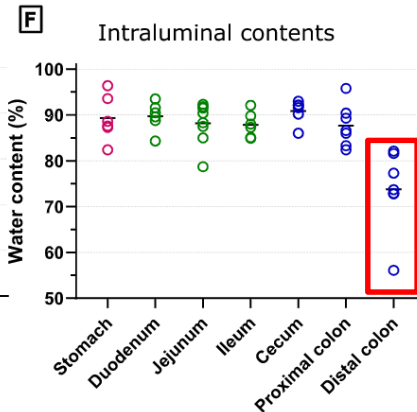
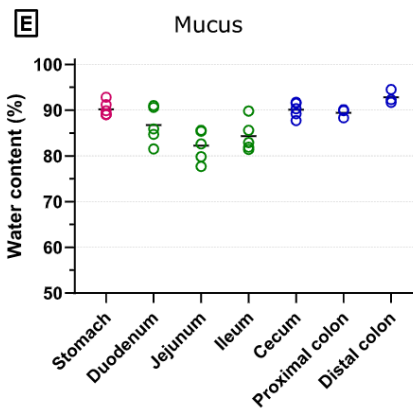
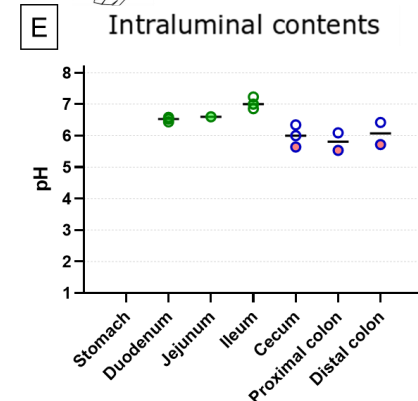
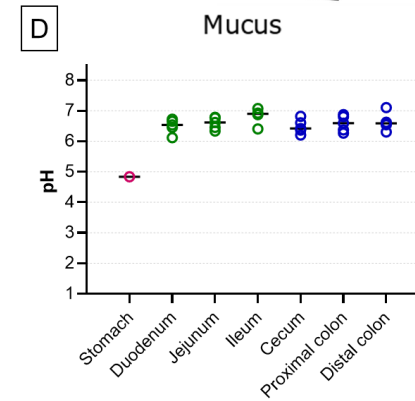
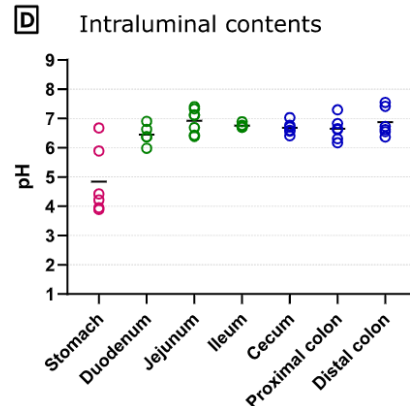
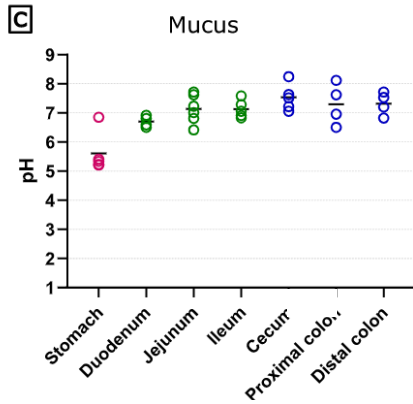
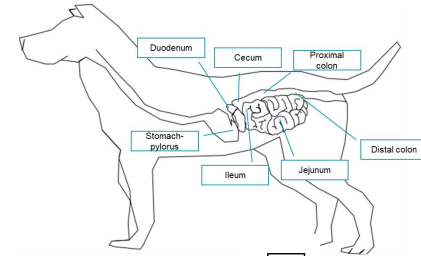
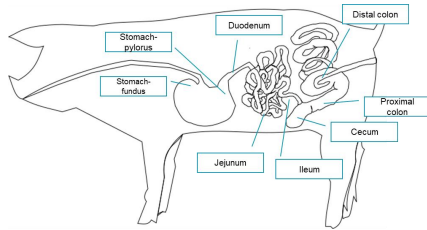


## Species differences:

Much greater yield – and easier to collect – mucus from colon of dogs as compared to pigs (0.3-1.9% whereas dogs typically range between 5-10%).

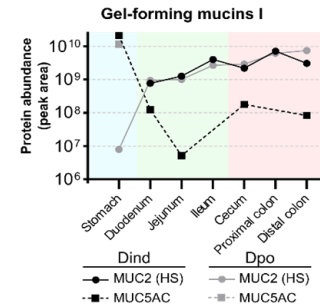
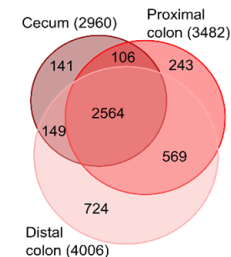
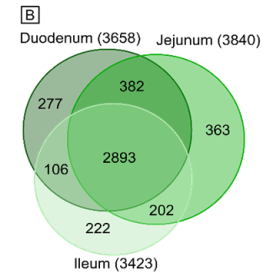
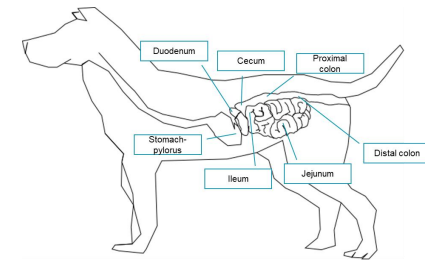
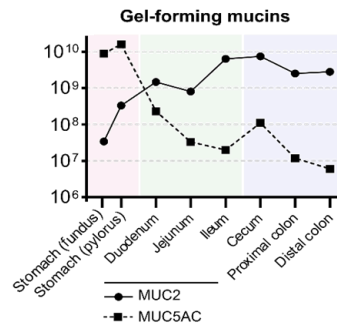
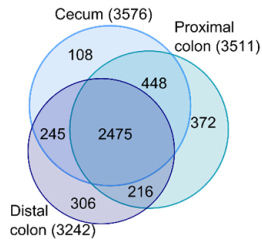
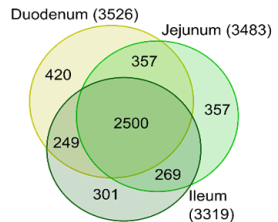
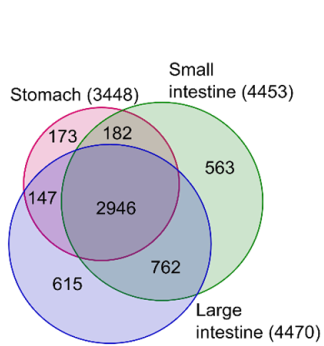
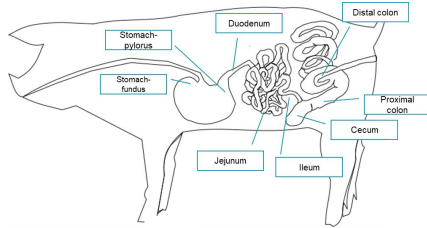


# Physiological properties –pH and water content

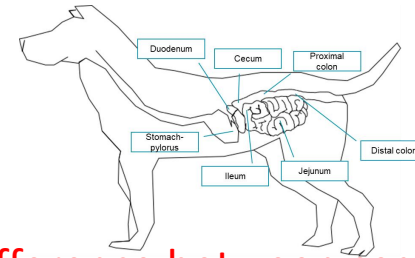
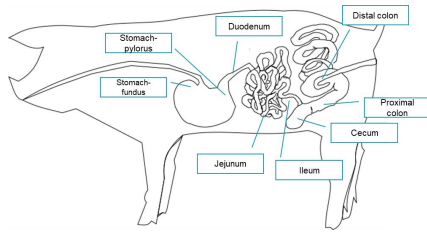




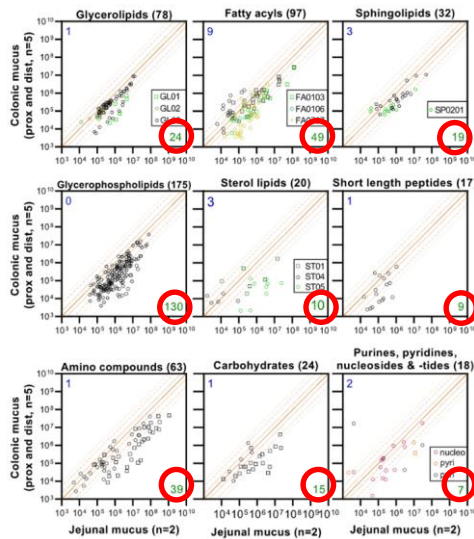
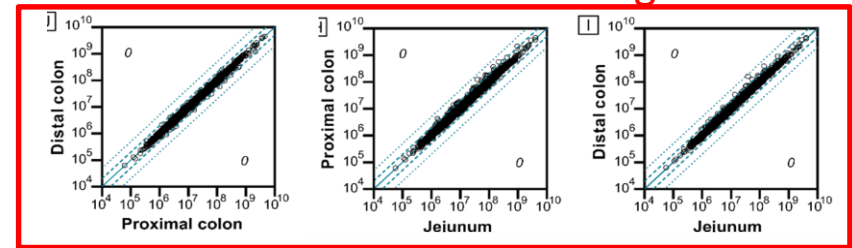
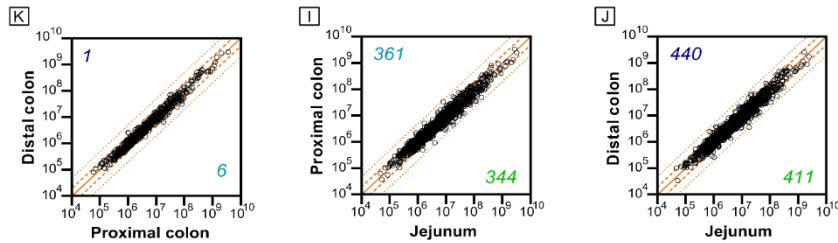
# Composition – global proteomics



# Composition – targeted proteomics, lipidomics and metabolomics

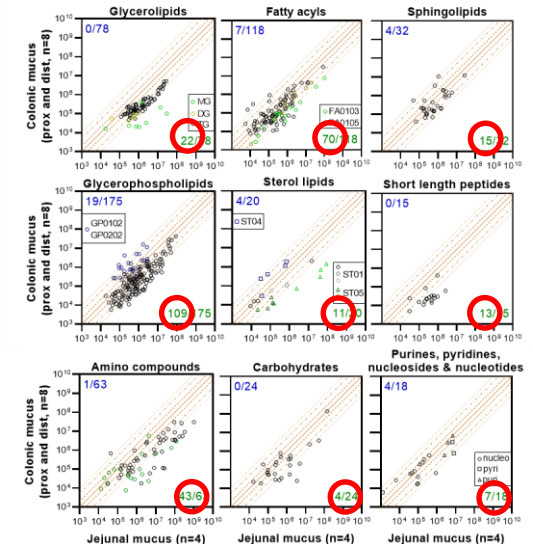


No difference between segments



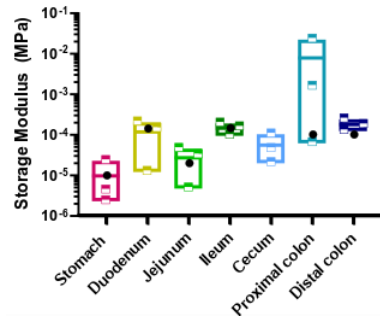
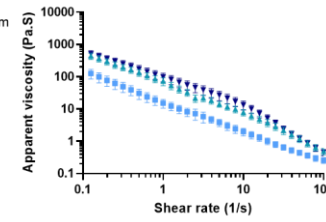
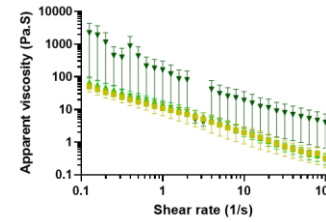
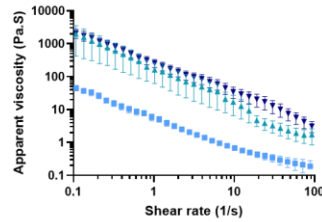
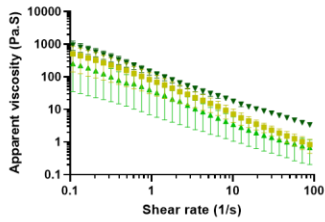
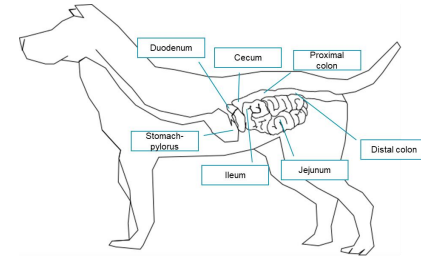
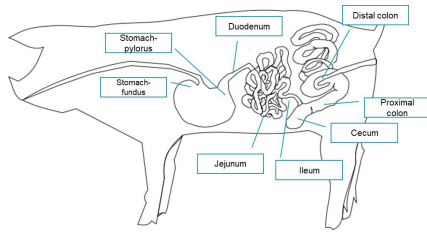
Numbers identify statistically significant different amounts of proteins, lipids, carbohydrates etc between segments

Always more lipids in jejunal mucus than colonic mucus

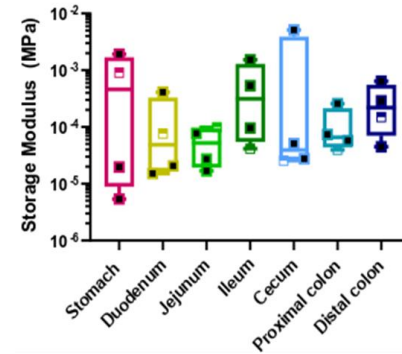




# Structural properties - viscoelasticity

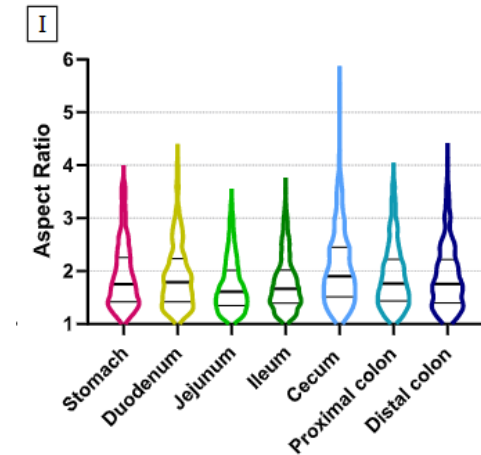
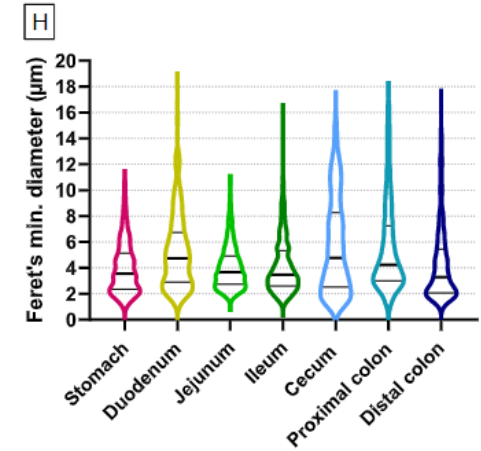
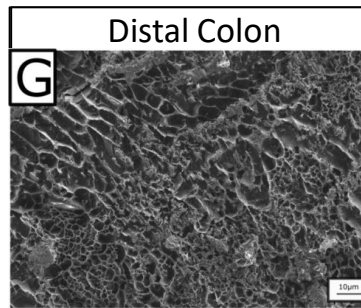
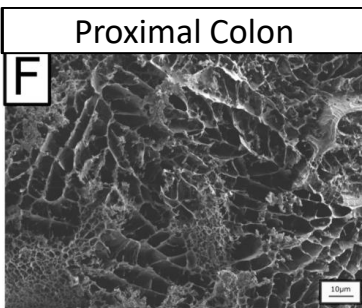
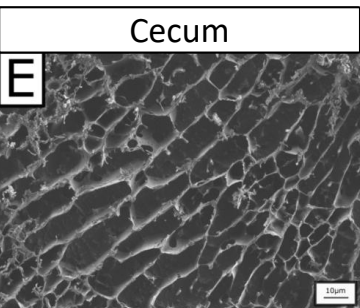
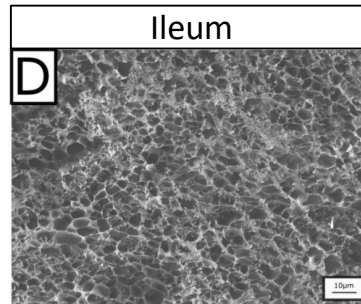
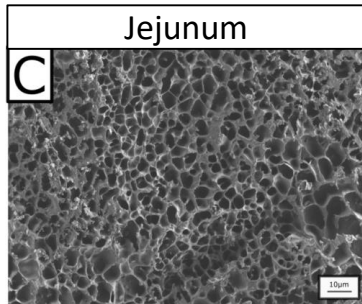
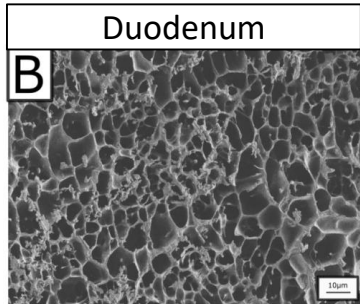
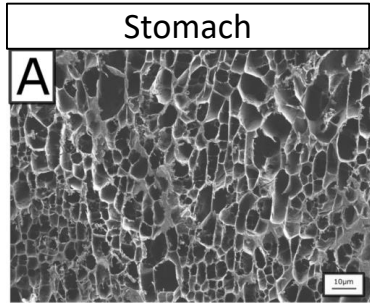
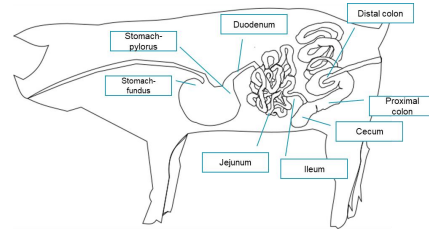


Pigs: Slightly higher apparent viscosity in general



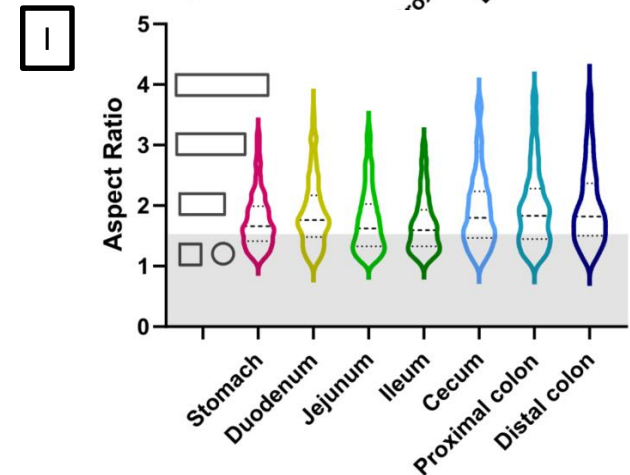
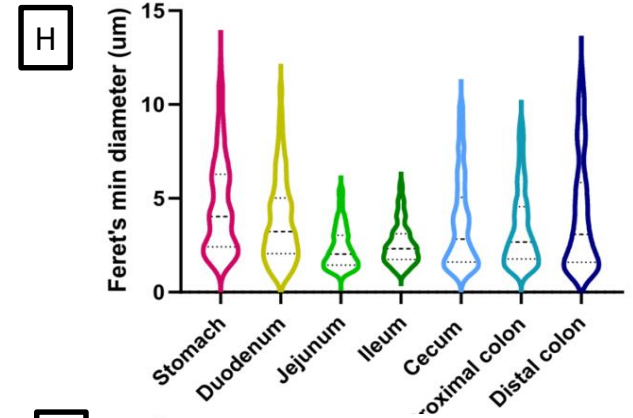
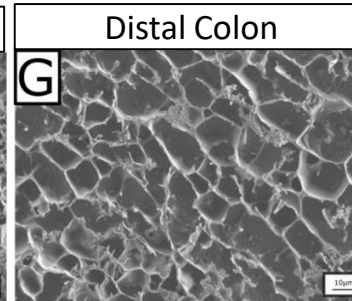
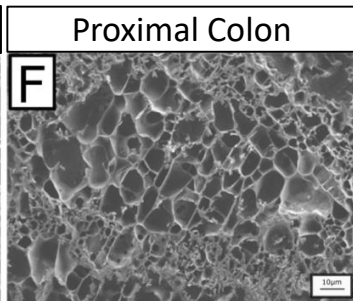
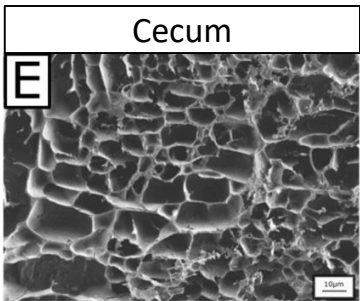
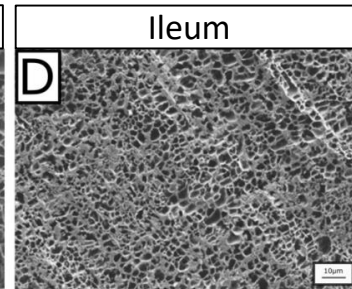
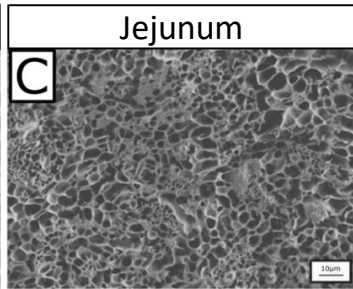
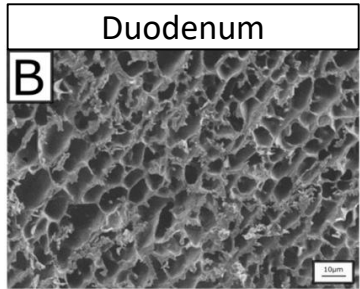
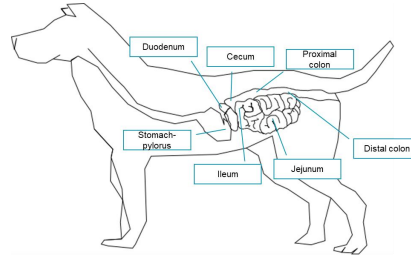
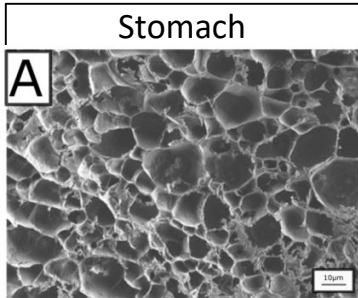


# Structural properties - porosity



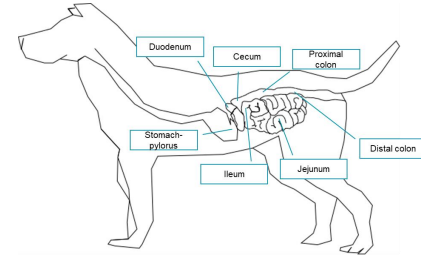
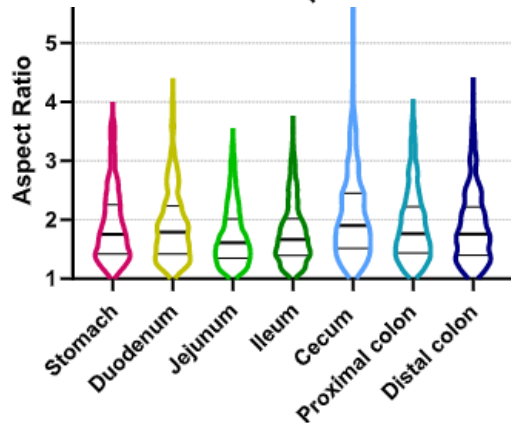
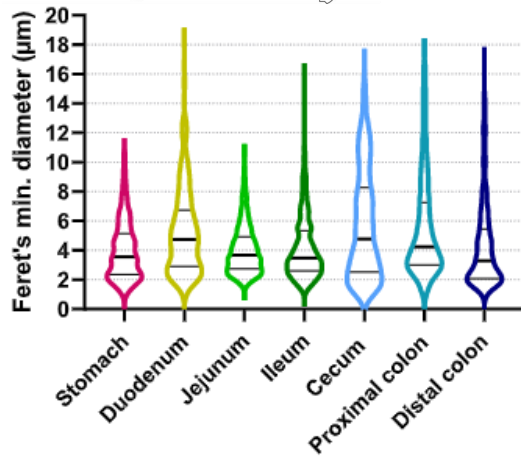
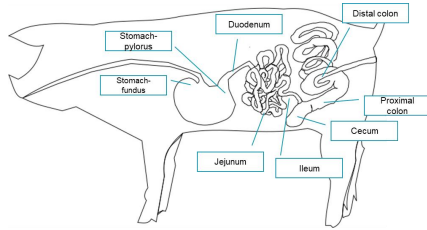


# Structural properties - porosity

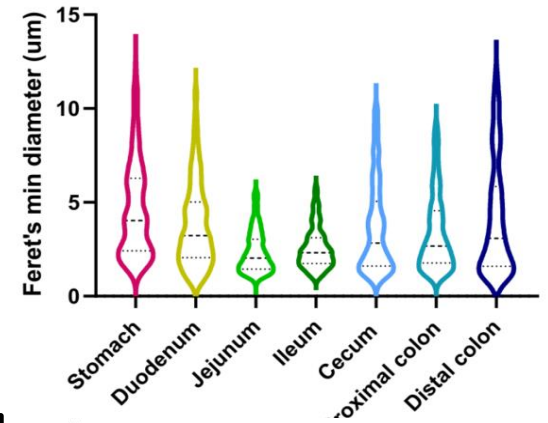




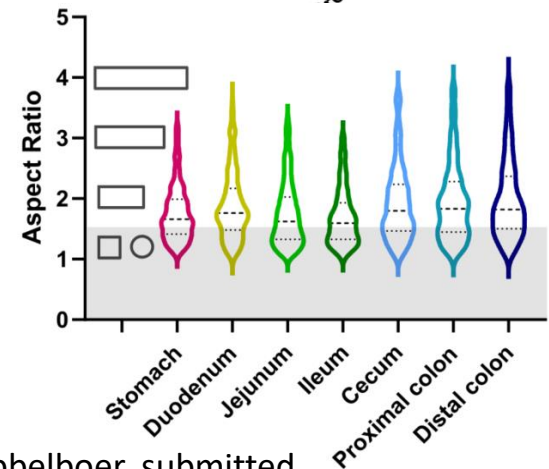
# Structural properties - porosity



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## Summary

- Extensive characterization of two major preclinical species have been undertaken
- The approach enables us to relate between GI regions as well as between species
- Regional differences observed in both species relate to pH, muc-2 presence, lipid content, rheological properties
- Differences between species relate to yield, water content and pore size, as well as some differences in protein and lipid content
- By making use of the obtained data artificial colonic mucus is now being develop. Our goal is to provide medium throughput 3R methods, generating data translatable to the human setting and useful in PBPK/PBB modelling.

# A perfect research environment for colonic research



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