THE UNIVERSITY OF MELBOURNE

Melbourne Veterinary School

The role of microbes in digestive function

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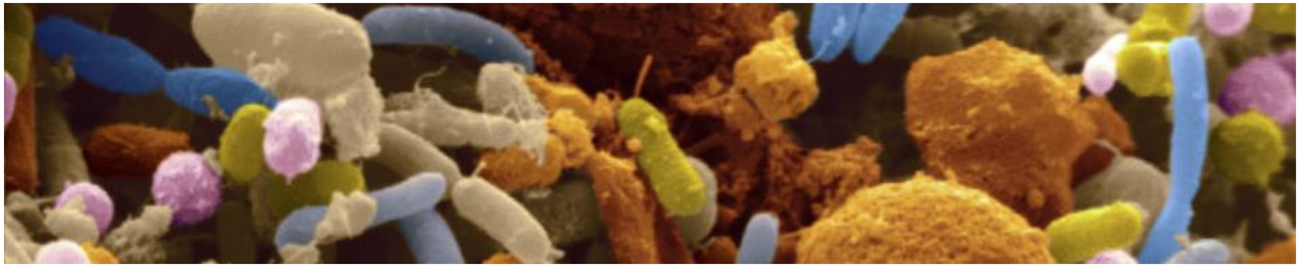
VETS30016 / VETS90120

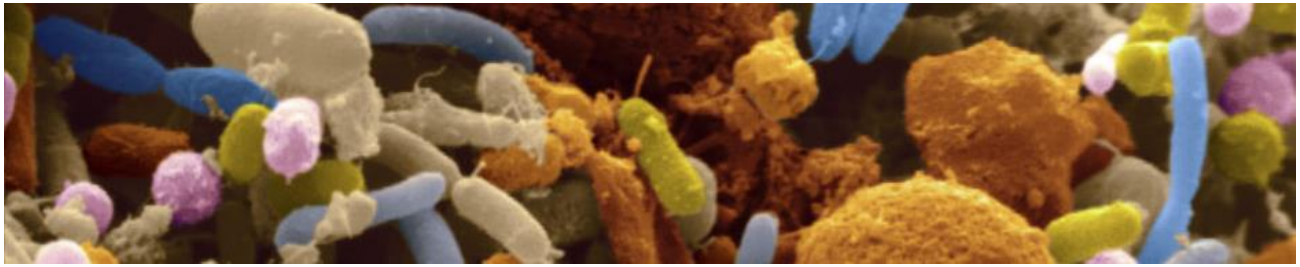
Intended learning outcomes

- Compare the roles of microbes in the digestive function of different animal species, and explain how disruption of the microbiota can result in disease
- Explain the role of microbes in the digestive processes of hindgut and foregut fermenting animals
- Discuss the relative advantages and disadvantages of:
    - Microbial digestion in the foregut
    - Microbial digestion in the hindgut



Terminology

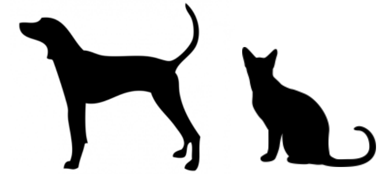
- *Microbes*
  - Microscopic organisms
  - Bacteria, protozoa, (archaea, fungi, viruses)
- *Microbiota*
  - Ecological community of microbes
  - Preferred term to *microflora*
- *Microbiome*
  - Genetic makeup of a particular microbial population



Introduction

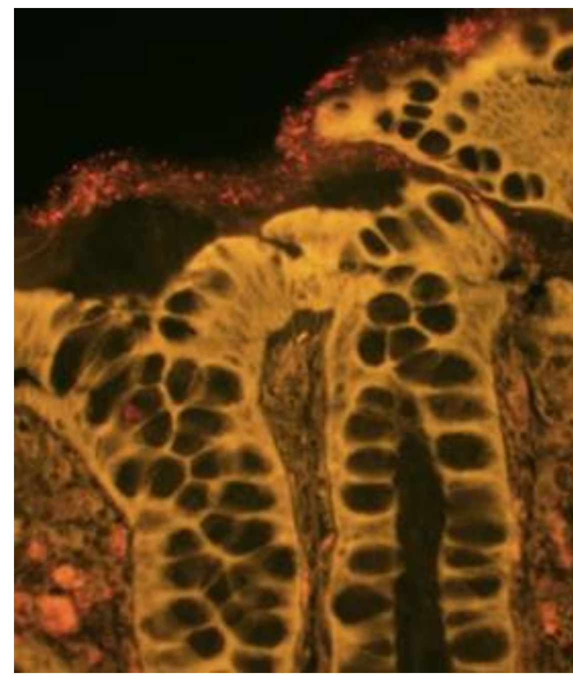
- Microbes play a crucial role in digestive processes
  - Digestive function
    - Particularly herbivores
    - Foregut and hindgut serve as fermentation chambers
  - Digestive health
    - Protective role against colonization
    - Promote enterocyte maturation

Carnivores



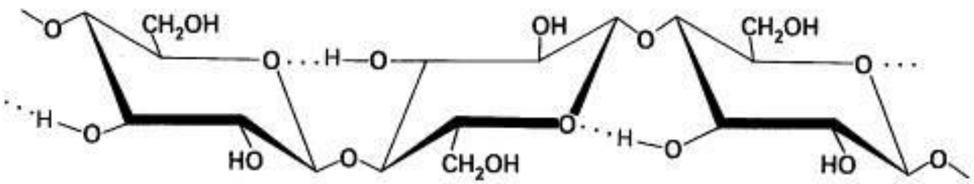
- Complex microbiota within colon
- Limited bacterial fermentation
    - Dietary fiber converted to VFAs
    - Some vitamin synthesis

- Promote health of intestinal mucosa
    - Proliferation of intestinal epithelial cells
    - Helps to prevent colonisation by harmful bacteria



Herbivores

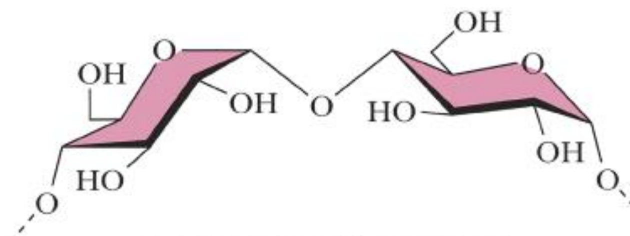
- Extract energy from plant cell wall (structural carbohydrates)
- Glucose locked up as CELLULOSE
  - Polymer of β-D-glucose attached by β-(1,4) linkages
  - Forms insoluble fibres
- Mammalian digestive enzymes cannot hydrolyse cellulose



Cellulose
(β-1,4-linkages)

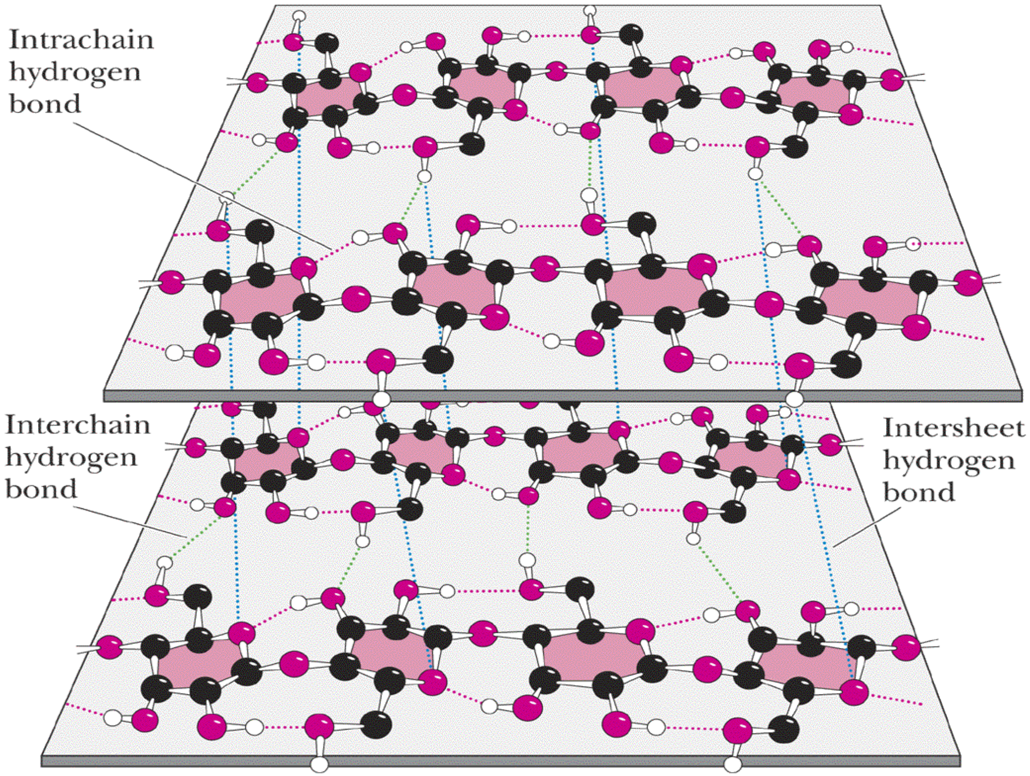
Schematic diagram showing the conformation of cellulose. The structure is stabilized by hydrogen bonds between adjacent glucose units in the same strand. In fibrils of cellulose, hydrogen bonds are formed between different strands as well.

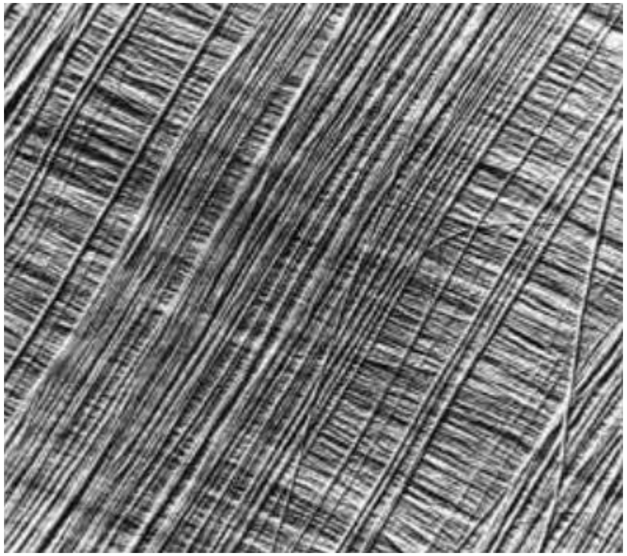
Starch



α-1,4-Linked D-glucose units

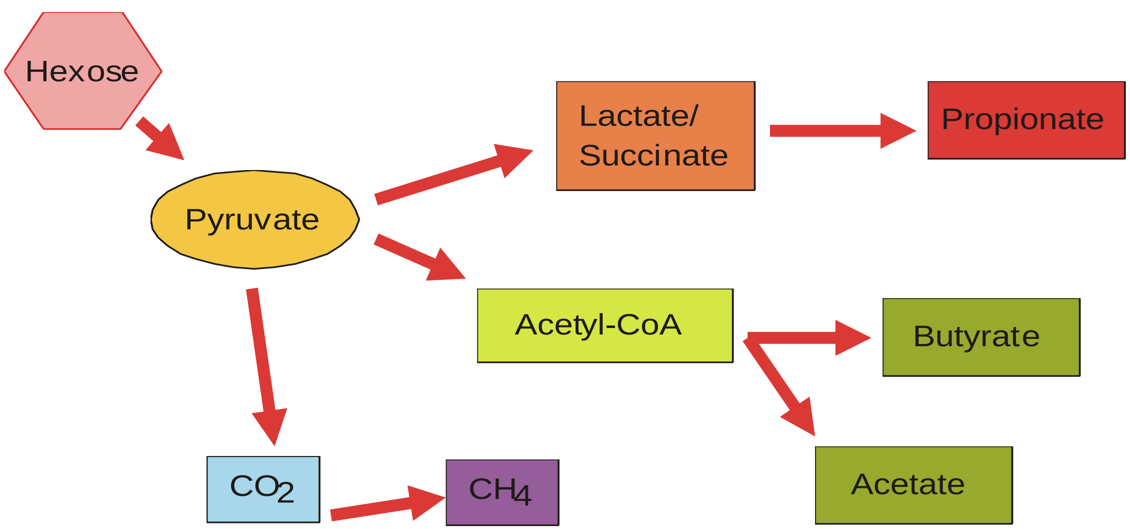
Structure of cellulose





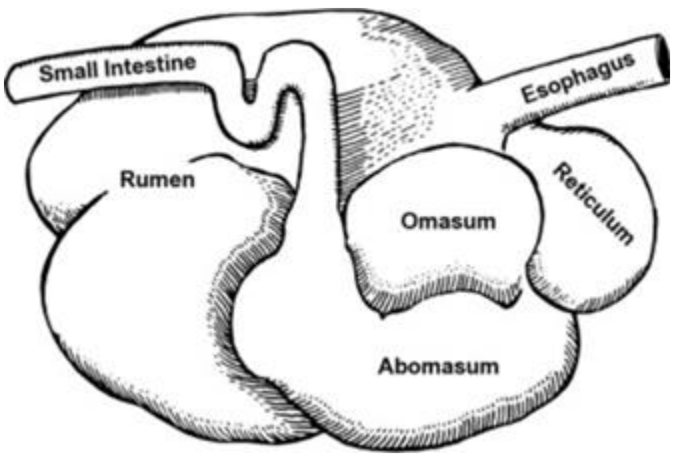
Fermentation

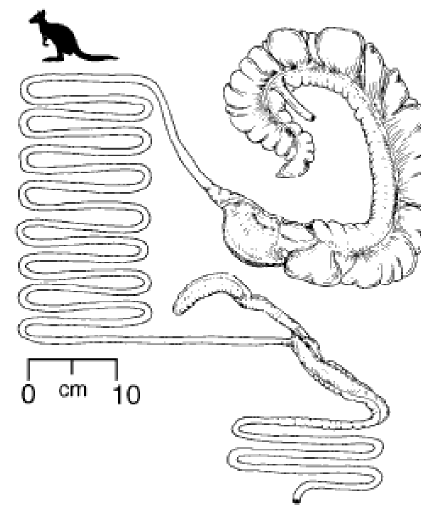
- Breakdown of carbohydrates by microbes under anaerobic conditions
- Breakdown product of sugars (hexoses) are volatile fatty acids (VFAs)
    - Absorbed and converted to energy substrates by host

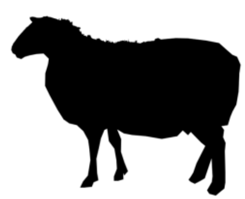


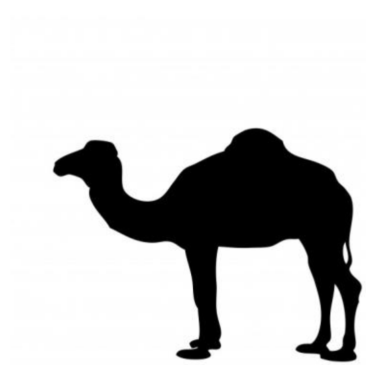
Foregut fermenters

- Microbial fermentation PRIOR to exposure to mammalian digestive enzymes



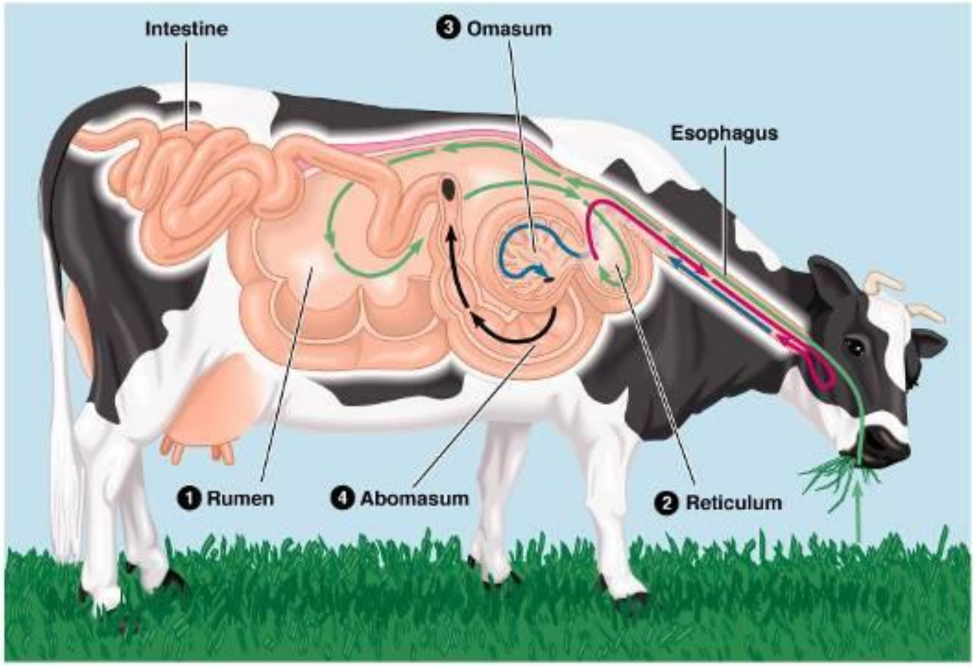






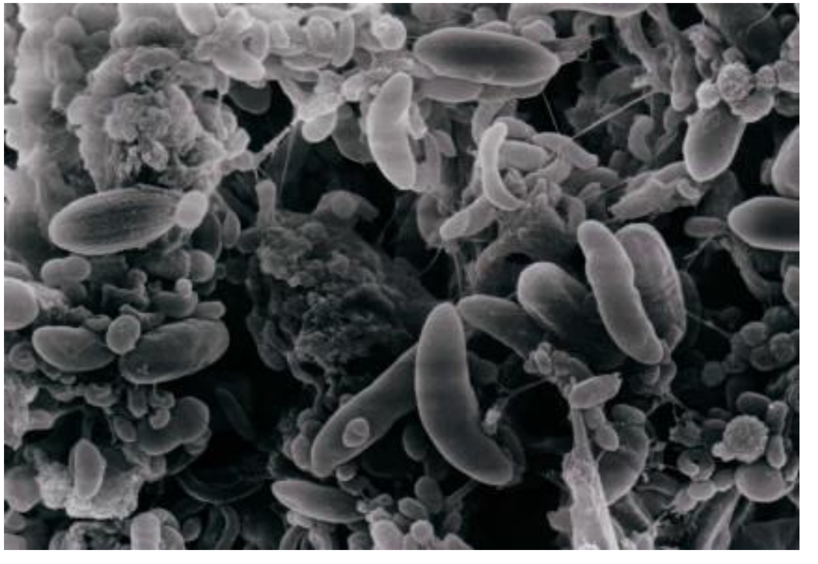
Ruminants

- Continuous culture system for anaerobic bacteria
  - (Plus protozoa and fungi)
- VFAs absorbed across the forestomach epithelium
  - Provide 60–80% of the energy needs of the animal



Rumen microbes

- 10^{10} to 10^{11} bacteria per gram content
  - Mostly attached to fibres of food material
  - ≈ 30 important bacterial species identified
  - Many species unidentified (>300)
    - Anaerobes
- (10^5 to 10^6 protozoa per gram, fungi)



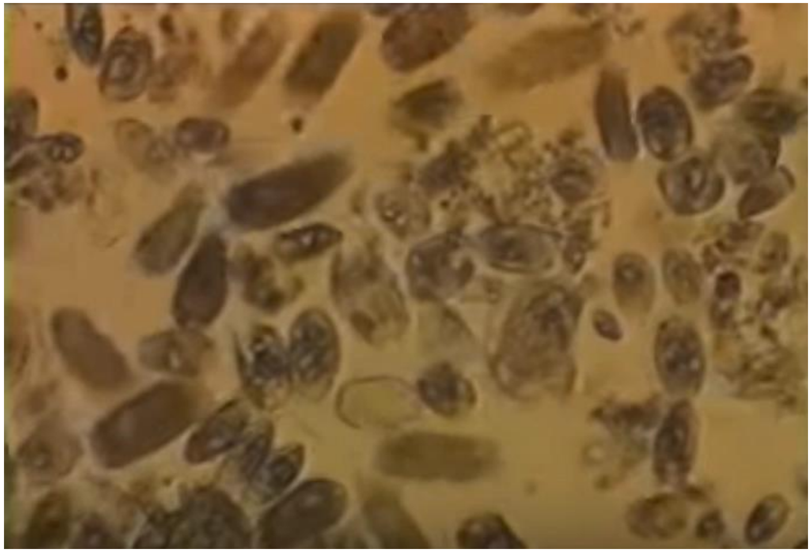
Bacterial symbiosis

- Bacteria produce substrates necessary for other processes
  - Some bacteria digest cellulose but not protein and *vice versa*
- Cellulose
  - e.g. *Ruminococcus* spp. (*R. albus*), *Bacteroides* spp.
- Starch and sugars
  - e.g. *Streptococcus spp. (S. bovis)*, *Lactobacillus* spp.
- Protein
  - e.g. *Bacteroides* spp. (*B. ruminicola)*, *Streptococcus* spp.

Rumen environment

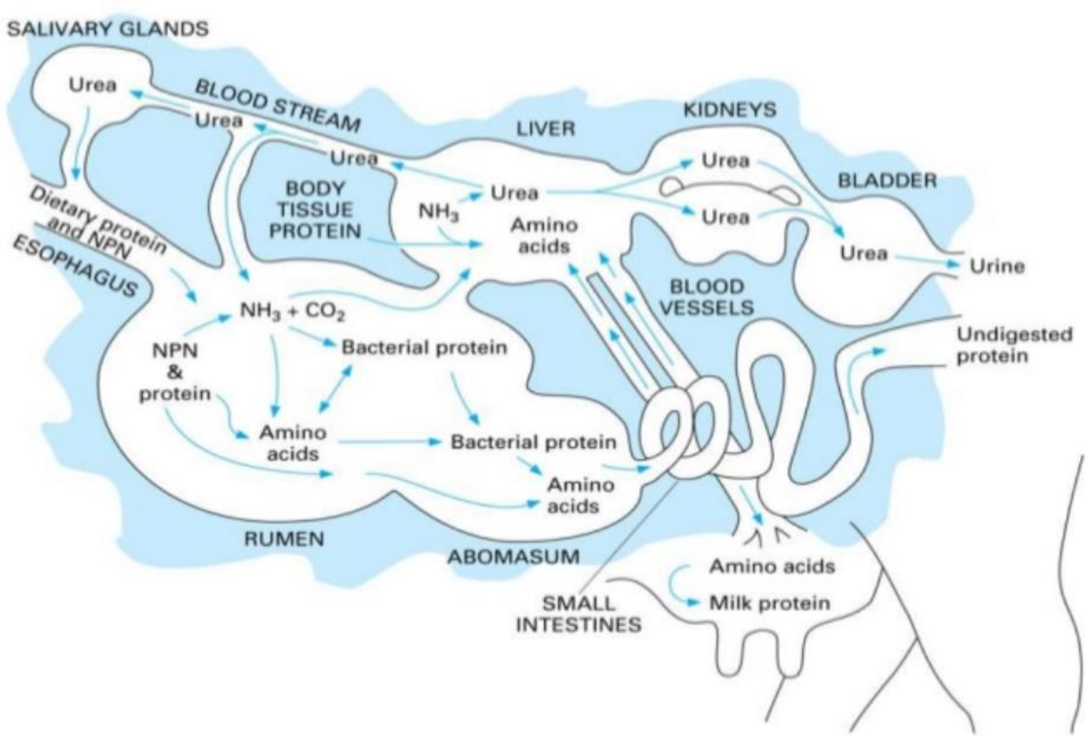
- pH ≈ 6.5
  - VFAs buffered by bicarbonate/phosphate in saliva
- Low oxygen
- Temperature
- Osmotic pressure
- Antibodies from saliva

Rumenocentesis



Bacterial protein metabolism

- Proteins converted to peptides by bacterial proteases
  - Used for energy or microbial protein
- Also utilise non-protein nitrogen – ammonia, urea
- Most protein reaching the small intestine is microbial



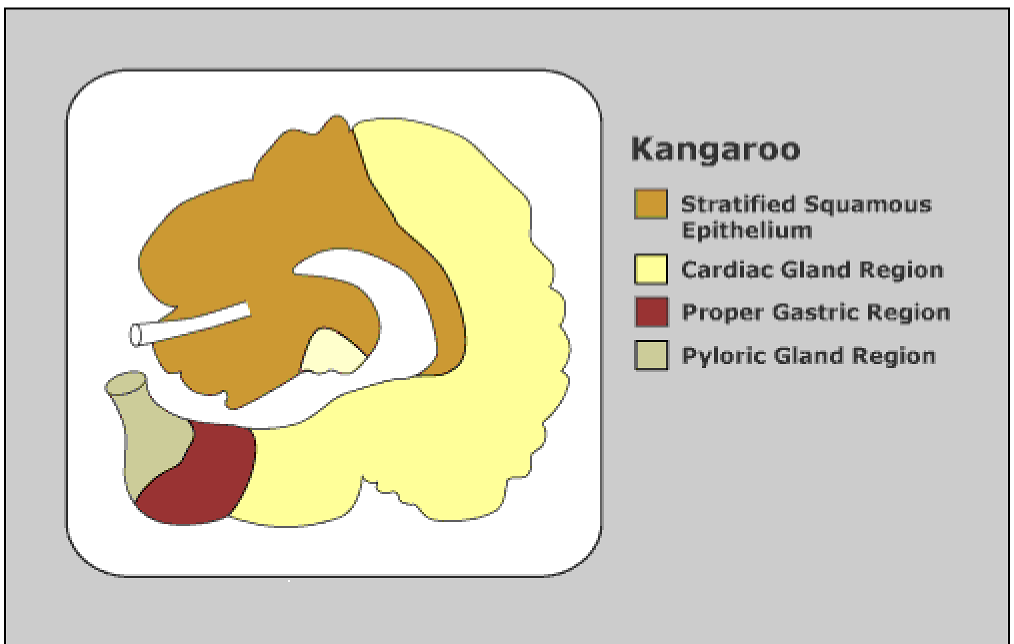
Ruminant hindgut

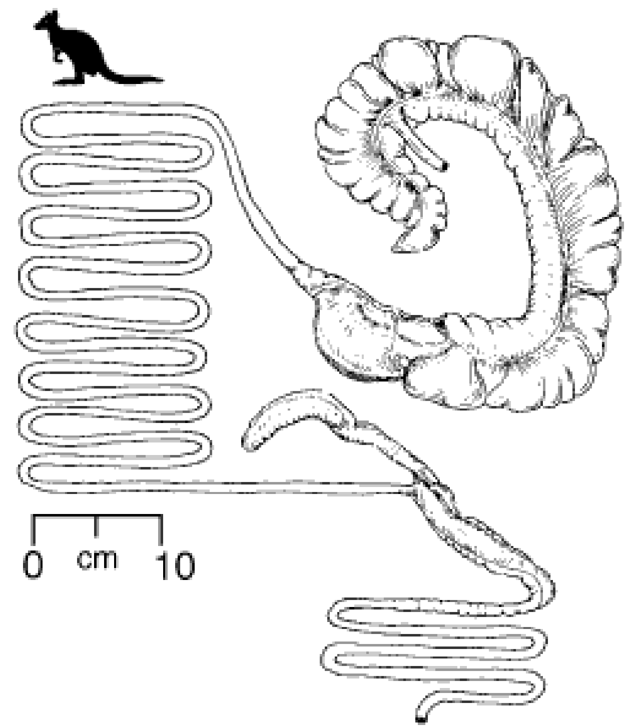
- Colon and caecum also sustain significant microbial populations
- VFA production and absorption
- Further bacterial action undigested fibre
    - (Very fibrous forage)

Non-ruminant foregut fermenters

e.g. Kangaroos

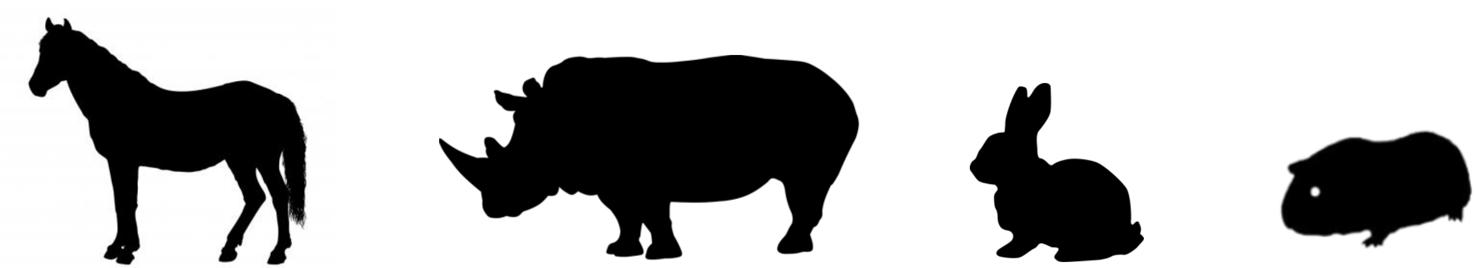
- Not a true ruminant
- Greatly expanded forestomach
- Very little methane production



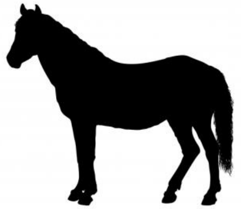


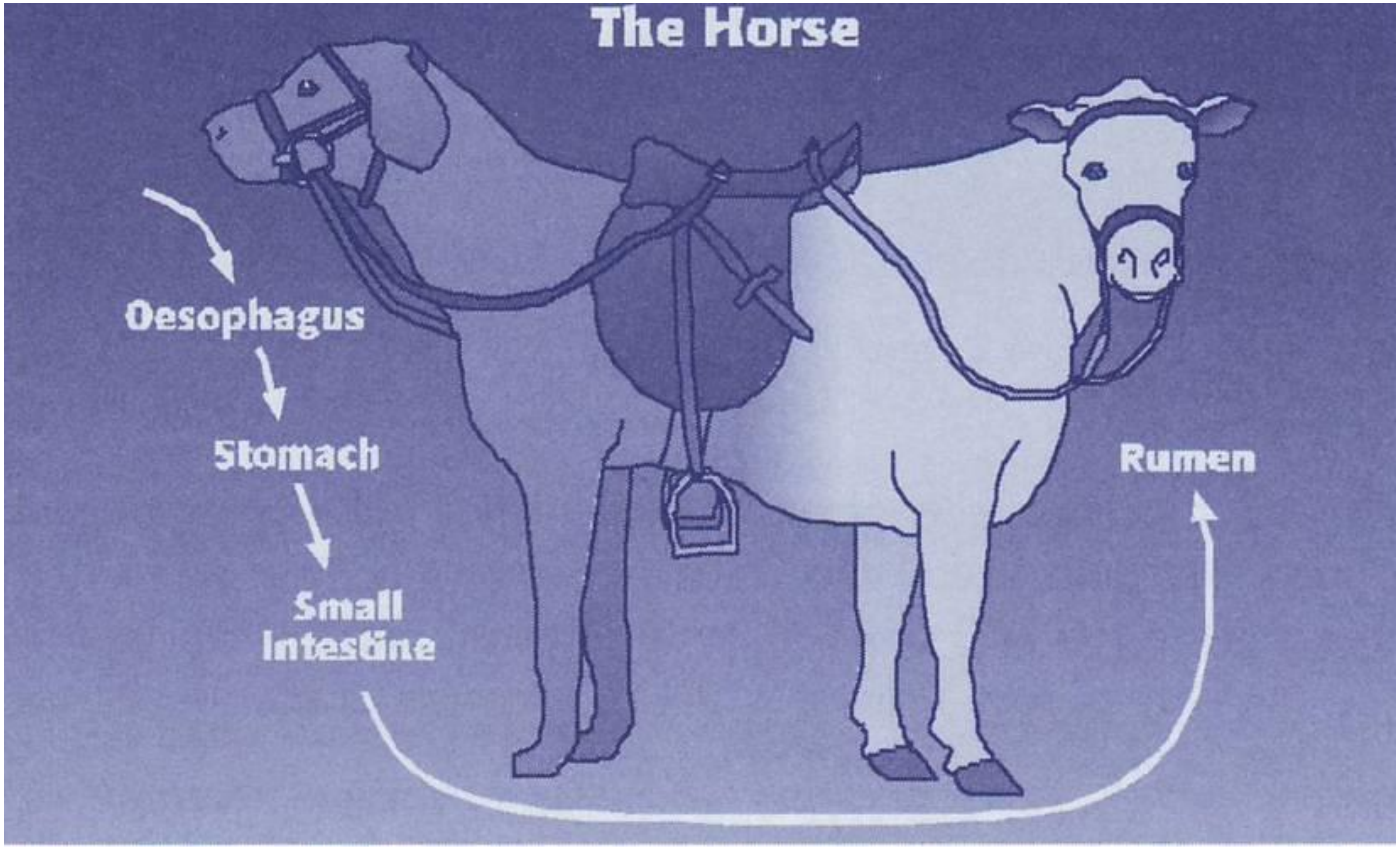
Hindgut fermenters

- Access to feed/nutrients before microbes
  - Good quality proteins and sugars digested first
  - Similar to carnivores and omnivores
- Mainly indigestible fibre acted on by bacteria
  - Also other carbs not hydrolysed by mammalian enzymes
  - e.g. fructans, pectins, β-glucan, resistant starch
- Not as efficient at digesting fibre as ruminants
  - Digesta not held in GI tract for sufficient time

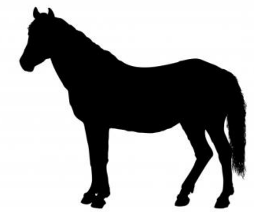


Horse

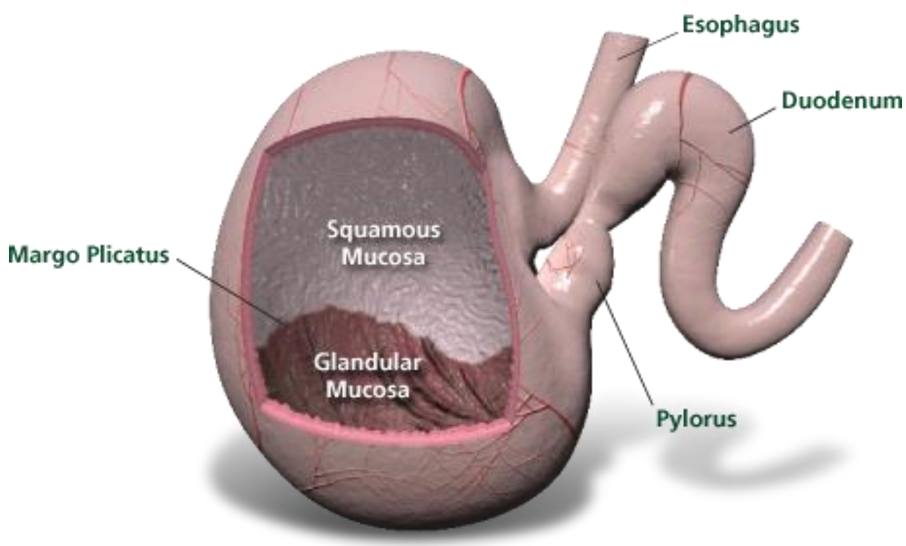




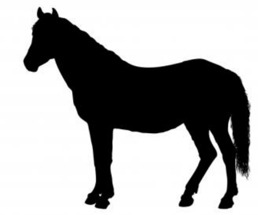
Bacteria in the equine stomach



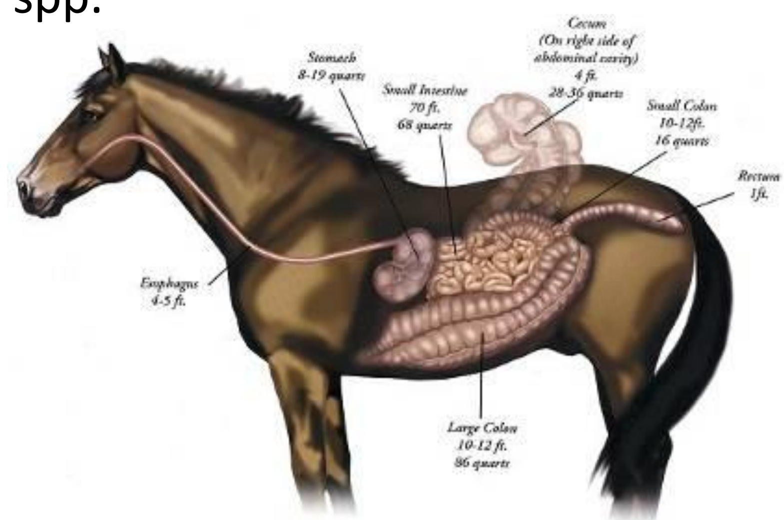
- Limited fermentation in the *saccus caecus*
- 10^8 to 10^9 bacteria per gram ingesta
- Digestion of some starch and fructan carbohydrate to VFAs
    - Large starchy meals – gastric ulcers



Horse: Caecum and colon

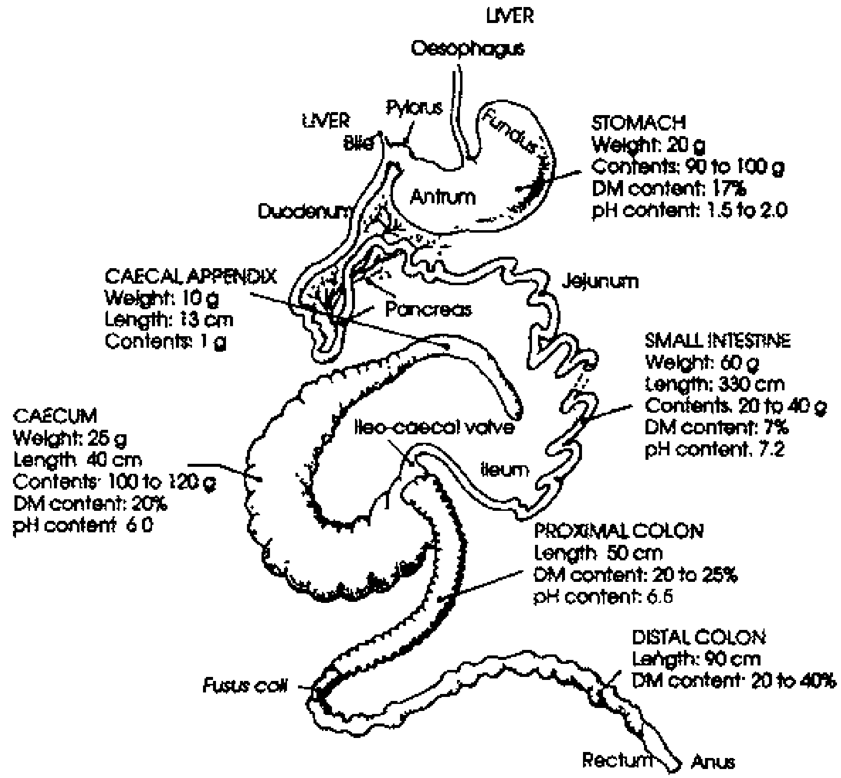


- Large volumes of ingesta
- Similar conversion of substrates to that of rumen microbes
- ≈ 10^{10} bacteria per gram content
- Cellulose
  - e.g. *Ruminococcus* spp., *Fibrobacter* spp.
- Starch and sugars
  - e.g. *Streptococcus* spp., *Lactobacillus* spp.
- Populations always changing
  - Composition of diet
  - Meals vs grazing



Rabbits

- Hindgut fermenters
- Large caecum (40% of intestinal contents)

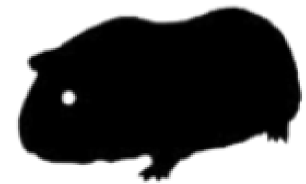


Note: Numerical values are those observed in the New Zealand White breed, aged 12 weeks, fed a complete balanced pelleted feed.

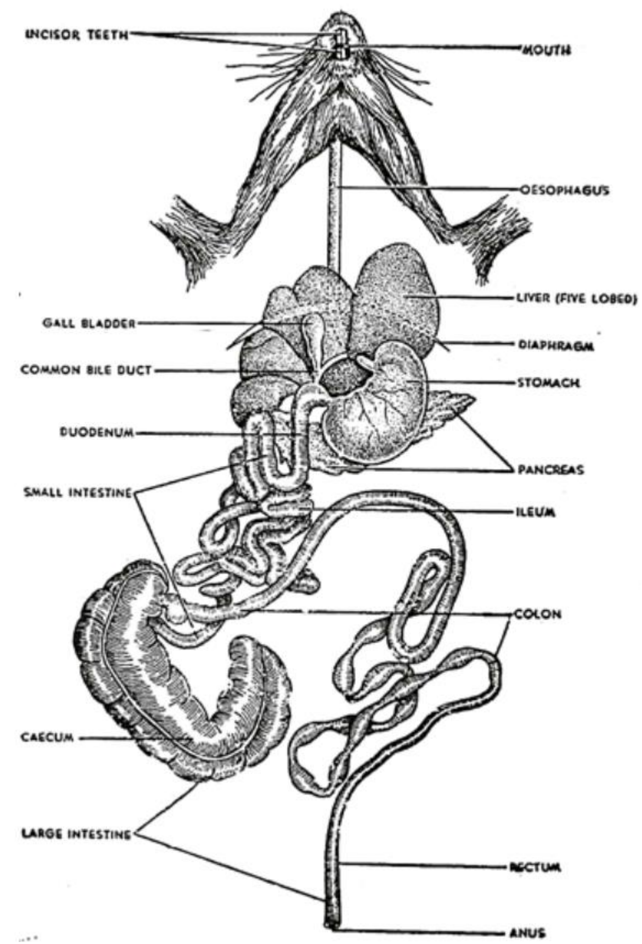
Caecotrophy

- Some products of hindgut digestion will not be absorbed
- Specialised type of coprophagy – consumption of faeces
- Caecotrophs
  - Contain microbes, amino acids, VFAs, vitamins
  - Mucous coating

Guinea pigs

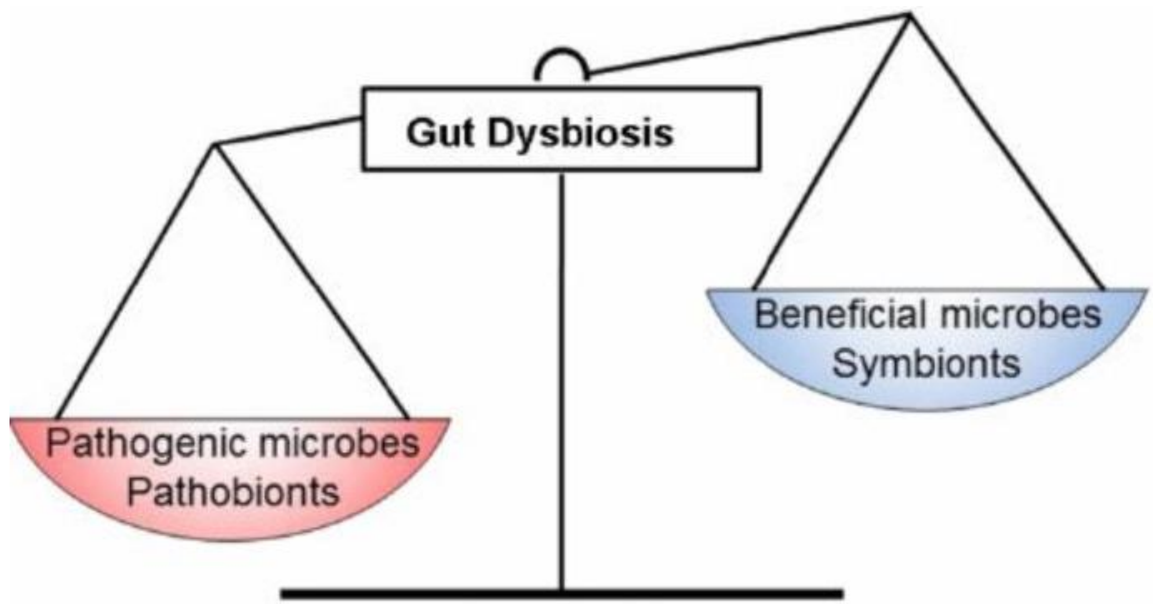


- Also caecotrophic
- Very large caecum
  - 65% of GI contents
- Gut flora contains many similar types of bacteria to rabbit & horse

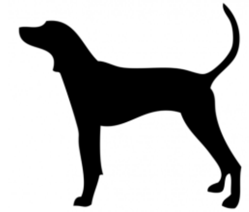


Disturbances of intestinal microbiota

- Balance between different microbes



Inflammatory bowel disease



- Canine IBD associated with dysbiosis
  - Microbiome requires further study

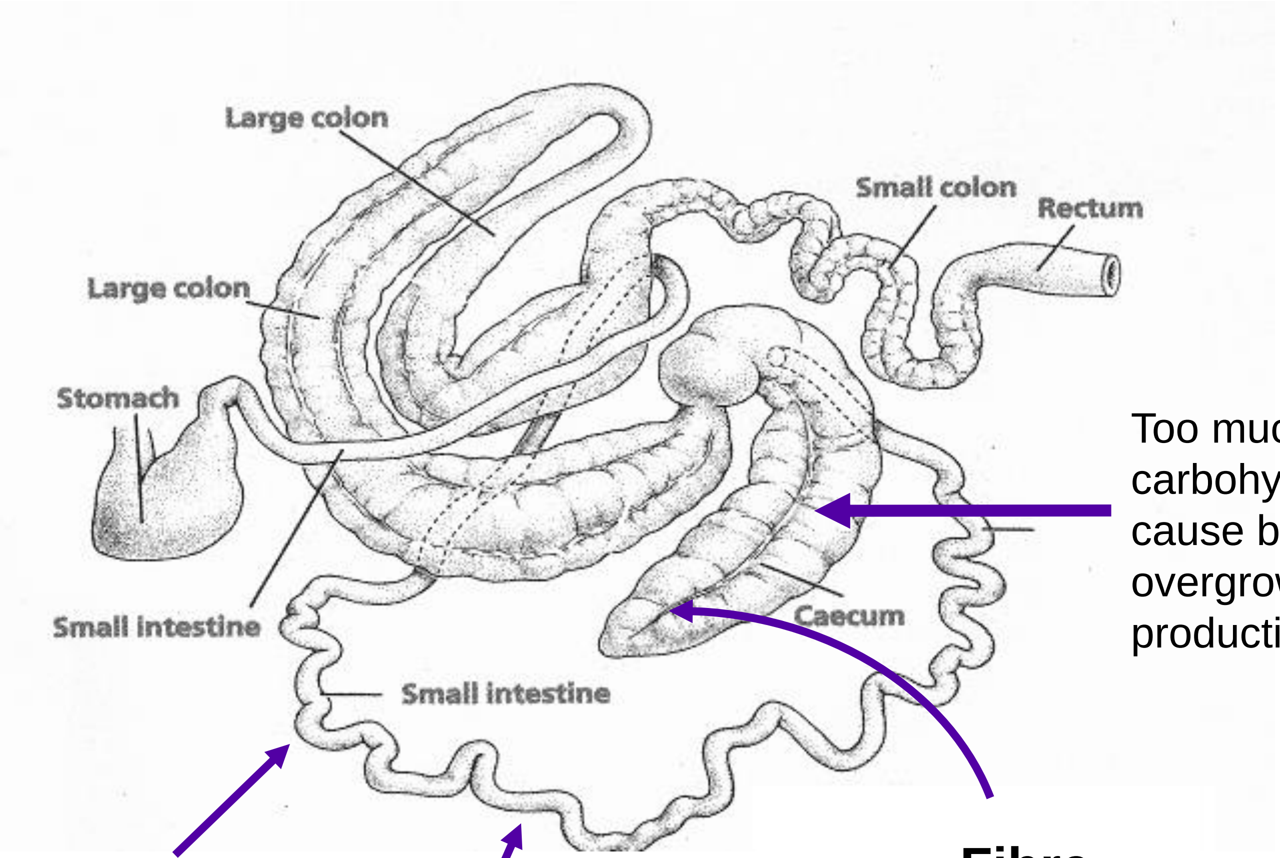
Therapies for dysbiosis?

- Probiotic
  - Formulation of live beneficial whole bacteria
  - Various licensed products
- Prebiotic
  - Substrate that promotes growth of beneficial bacteria
  - e.g. psyllium, pectin, beta-glucan

Ruminal acidosis

- Excess dietary hydrolysable carbohydrate
- *Streptococcus* spp. and *Lactobacillus* spp. ferment starch
  - Lactic acid
- Acute form
  - pH < 5.5 → other bacteria die, acidic damage to rumen mucosa → fluid movement into rumen → dehydration
- Subacute form
  - Rumenitis, dysbiosis
  - Decreased energy production and absorption
  - Reduced growth, illthrift

Carbohydrate overload



Too much fermentable carbohydrate may cause bacterial overgrowth and toxin production

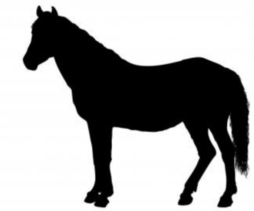
Sucrose, Fructose

Starch

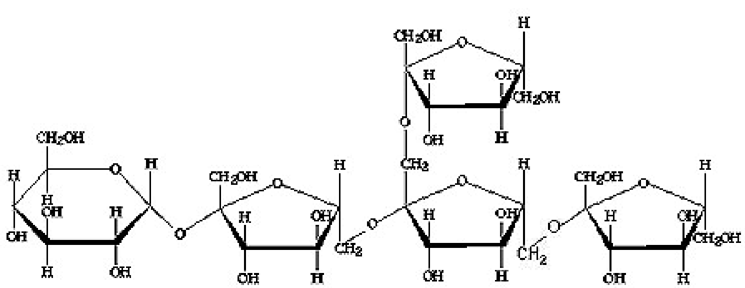
(>0.4% bwt)

Fibre,
Fructans,
Starch overload

Fructan carbohydrates

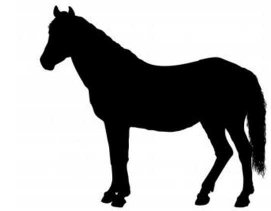


- Storage form of carbohydrate in stems of grasses
- Polysaccharides of one glucose with many fructoses
- Both β-(2 → 1) and β-(2 → 6) linkages
    - Resistant to mammalian enzymes
    - Susceptible to bacterial enzymes
- Lush grass high in fructans (e.g. spring) can cause hindgut disturbances



Fructan

Carbohydrate overload



- *Streptococcus* spp. and *Lactobacillis* spp. ferment starch or fructan carbohydrates to lactic acid
  - Other bacteria die
- Numbers increase 100 – 1000 X in few hours
- pH drops, damage to caecal mucosa
  - Bacterial toxins released into circulation
  - Horses exquisitely sensitive to effects of bacterial toxins

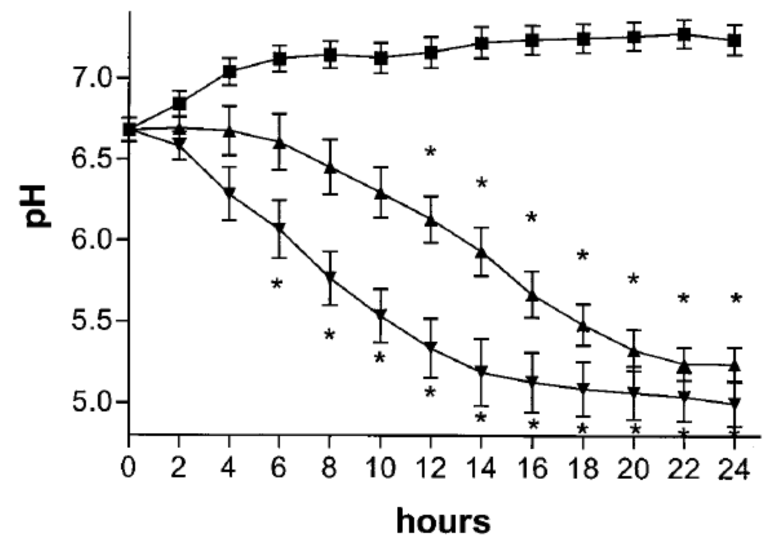
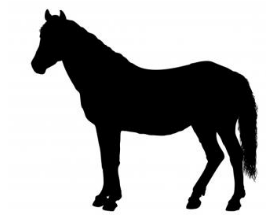
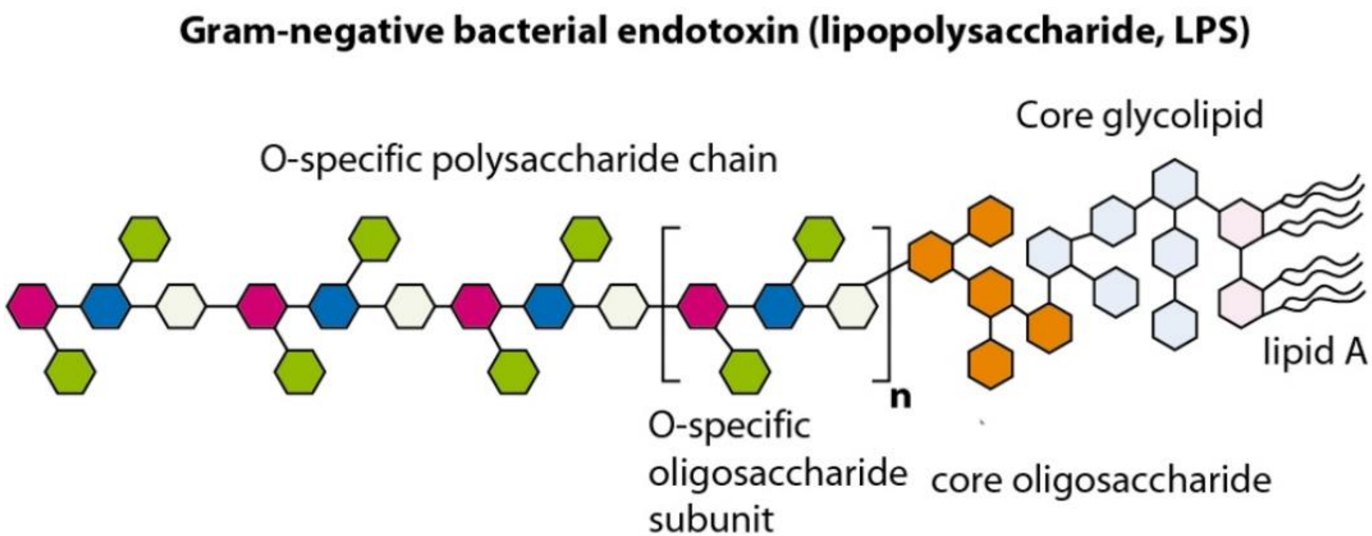


Figure 1. Effects of carbohydrate overload on the pH of equine cecal contents incubated anaerobically in vitro.

Lipopolysaccharide (endotoxin)

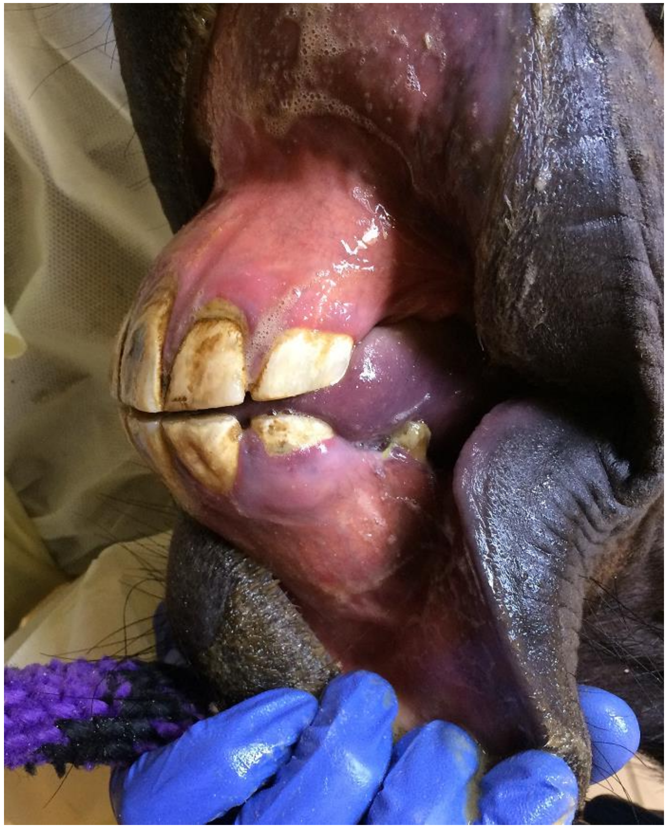


- Outer membrane of Gram-negative bacteria
    1. O-antigen (O-polysaccharide)
    2. Core oligosaccharide
    3. Lipid A
- LPS (endotoxin) is highly antigenic and in the circulation will cause massive systemic inflammatory response

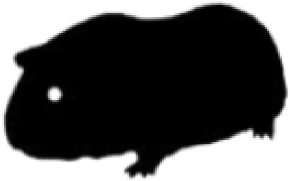


Endotoxaemia

Systemic inflammatory response syndrome (SIRS)

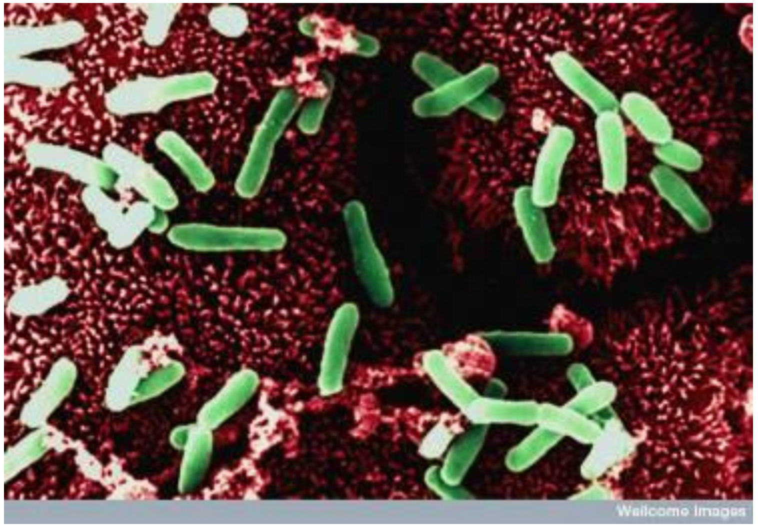


Enteritis in guinea pigs



- Hindgut microbiota disrupted by stress, illness
  - Intestinal stasis

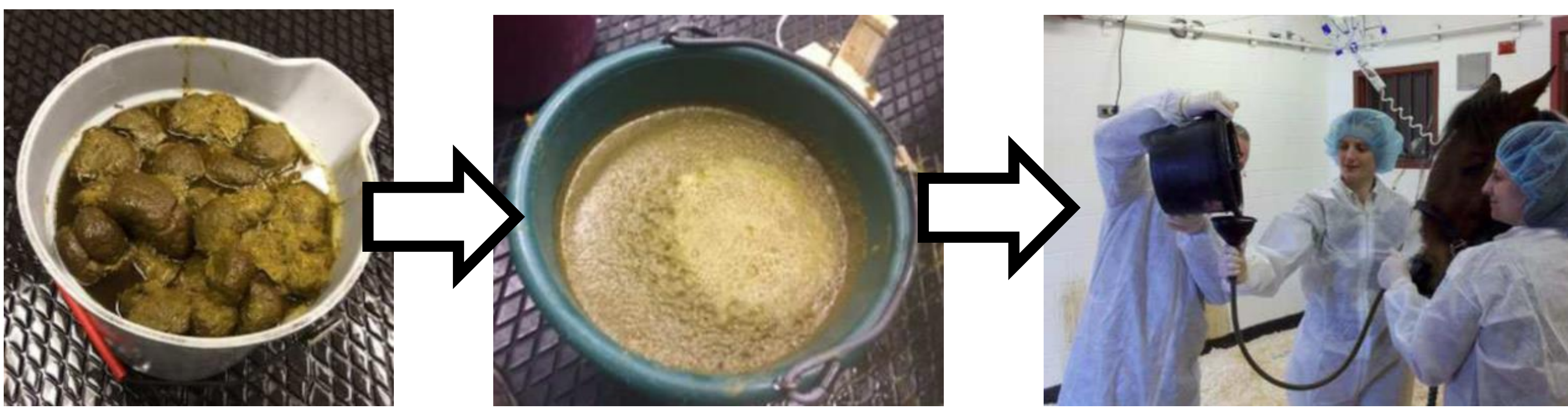
- Secondary to antibiotic therapy
  - e.g. Oral penicillin
  - Overgrowth of *Clostridium* spp.
  - Potent toxins
  - Diarrhoea and dysentery



Colour-enhanced scanning electron micrograph of rod-shaped Clostridium difficile clinging to gut microvilli

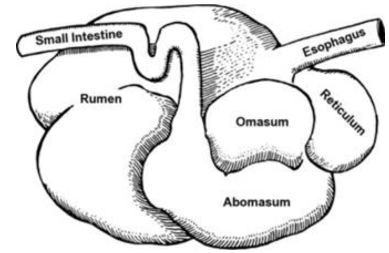
Transfaunation

- Process of transferring microbe-rich digestive contents from one individual to another
  - Rumen contents in cows
  - Faeces or caecal contents in horses
- Repopulate gut with healthy microbiota

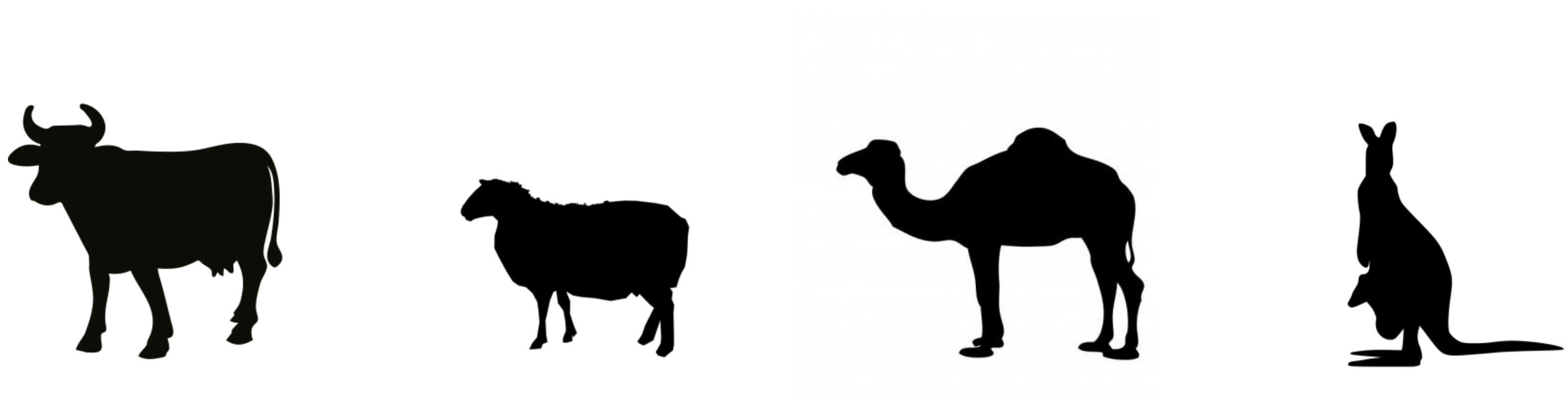


Mullen et al. *Equine Vet. Edu.*

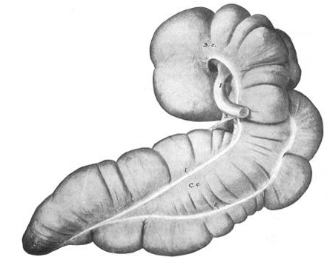
Summary: Foregut fermenters



- Can consume large amount of feed rapidly
- Higher efficiency at extracting nutrition from feed
  - Rumination, additional fermentation in caecum/colon
  - Advantageous when fibre is high, overall intake low
- Loss of easily available energy to fermentation
  - ‘Microbial processing cost’



Summary: Hindgut fermenters



- Need to eat small amounts frequently
  - Only one opportunity to chew feed
- Digestion occurs before reaching fermentation chamber
  - Dietary protein, fats and non-structural carbohydrates utilised similarly to carnivores/omnivores
  - No 'microbial processing costs'
  - Benefit from higher quality diet
- Some valuable nutrients & microbial population lost in faeces
  - Horses digest ≈ 85% cf. cattle
  - Some species have specialised adaptation – caecotrophy