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Prebiotic with special emphasis on butyrogenic carbohydrates and the effects on gut function

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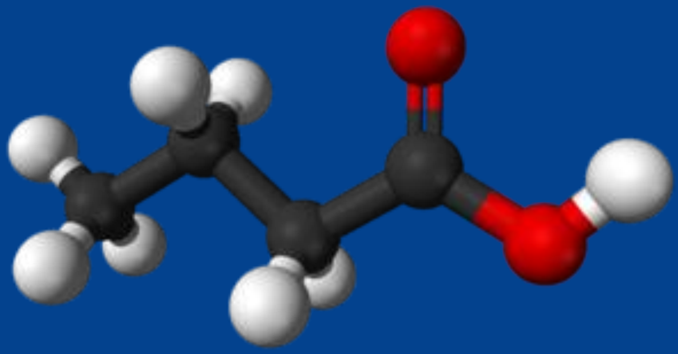
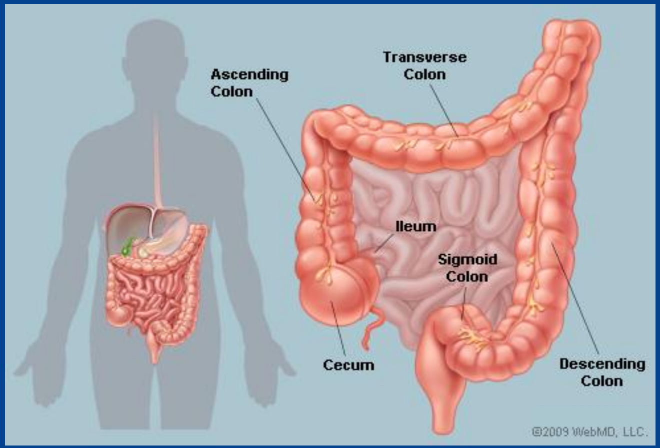
24. Hüsenberger Gespräche 6.-8. Juni 2012





Points to be addressed

- Introduction
- Prebiotics and butyrogenic carbohydrates
- Fermentation in the colon and factors influencing butyrate production
  - Substrate
  - Microflora
  - Cross-feeding
- Butyrate and gut function
- The ButCoIns project
- Summary

Introduction: SCFA and colonic health

- Colonic health has been increasing linked to maintaining overall health and reducing the risk of various diseases by changes in diet and lifestyle
- Importance in this connection is the substrates that reach the large intestine – prebiotics, dietary fibre, and other dietary components that target the colon and affect its environment, enhancing among other things the production of SCFA
- Butyrate is the acid recognised to have the most potent effect on colonic health

Definition of prebiotics

"A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota"

Food and Agriculture Organization of the United Nations (2007)

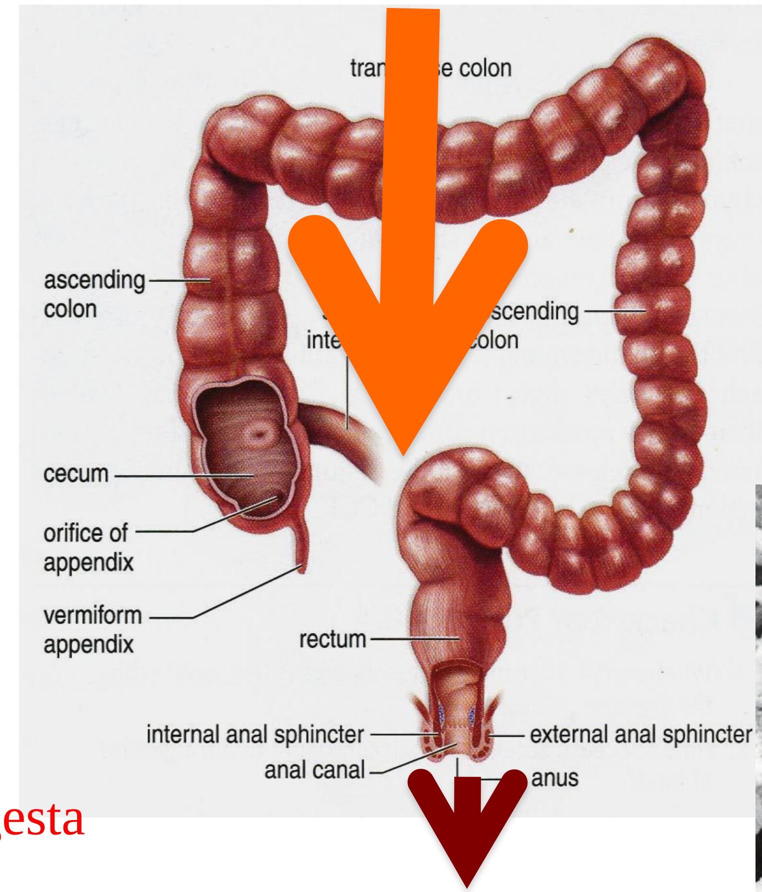
Definition of butyrogenic carbohydrate

"A butyrogenic carbohydrate is a prebiotic that selectively stimulate the production of butyrate, i.e. increase the proportion of butyrate"

Own working definition

The human colon – site for SCFA production

Ileal flow



Dimension:

154 (113-207) cm in length

1274 (731-2509) cm^2 in area

Content:

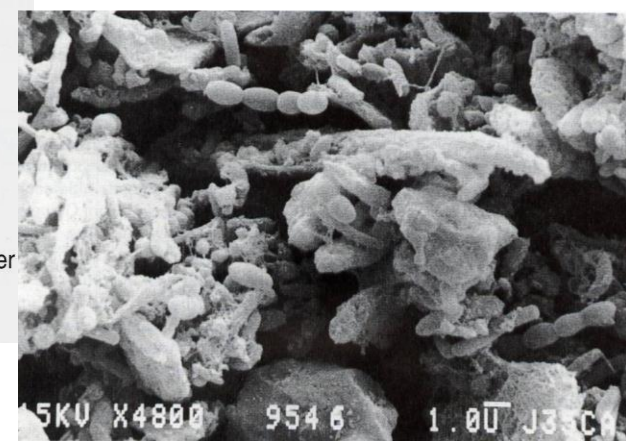
222 (58-904) g wet digesta

36 g DM and 186 g water

Bacteria:

10^{11}-10^{12} per gram biomass

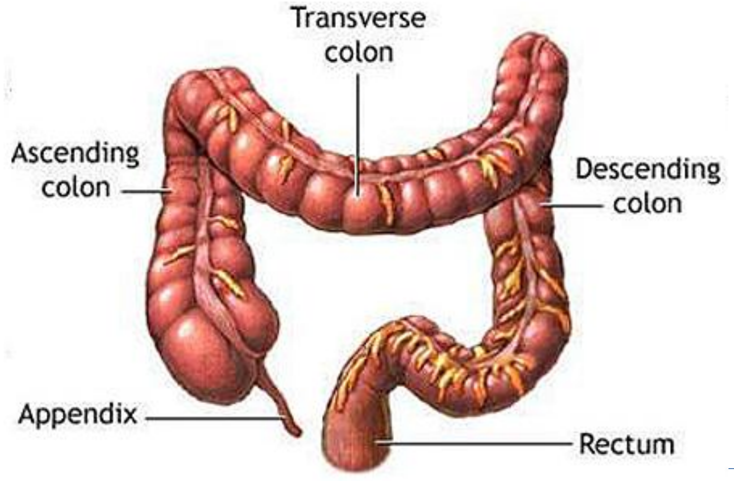
5-6 genera account for 99 % of biomass



Microbes in the colon

- Firmicutes (40-65%)
  - Lactubacillus / Enterococcus (0.01-1.8%)
  - **Clostridia cluster XIVa**
    - Rosburia / Eubacterium rectale
    - Eubacterium hallii
    - Ruminococcus obeum
  - Clostridia cluster IV
    - Faecibacterium prausnitzii
  - Clostridia cluster IX
  - Clostridia cluster XVI
- Bacteroidetes (15-35%)
- Actinobacteria (2-15%)
  - Bifidobacterium spp (2.5-5%)

10^{11}–10^{12} bacteria/g



Louis et al (2006); Arumugam et al. (2011)

Butyrate producing microbes in the colon

- Firmicutes (40-65%)
  - Lactubacillus / Enterococcus (0.01-1.8%)
  - **Clostridia cluster XIVa**
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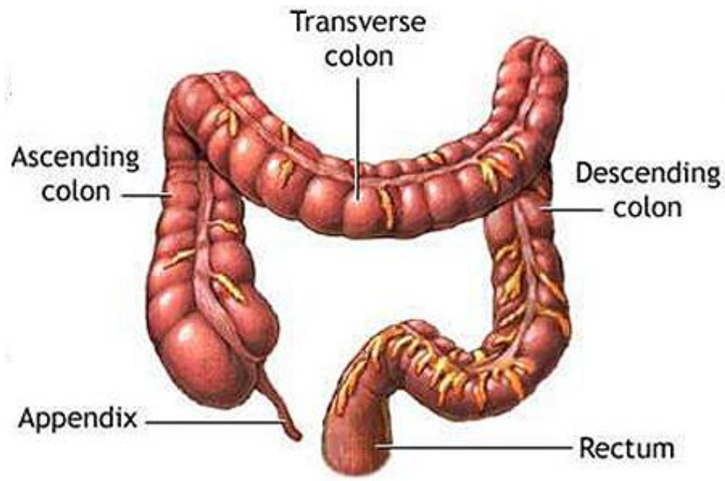

Louis et al (2006); Arumugam et al. (2011)

Regulatory factors influencing butyrate production

- Amount and types of substrate available
- Microbial composition
- Rate of degradation – pH
- Acetate ↔ butyrate exchange
- Retention time

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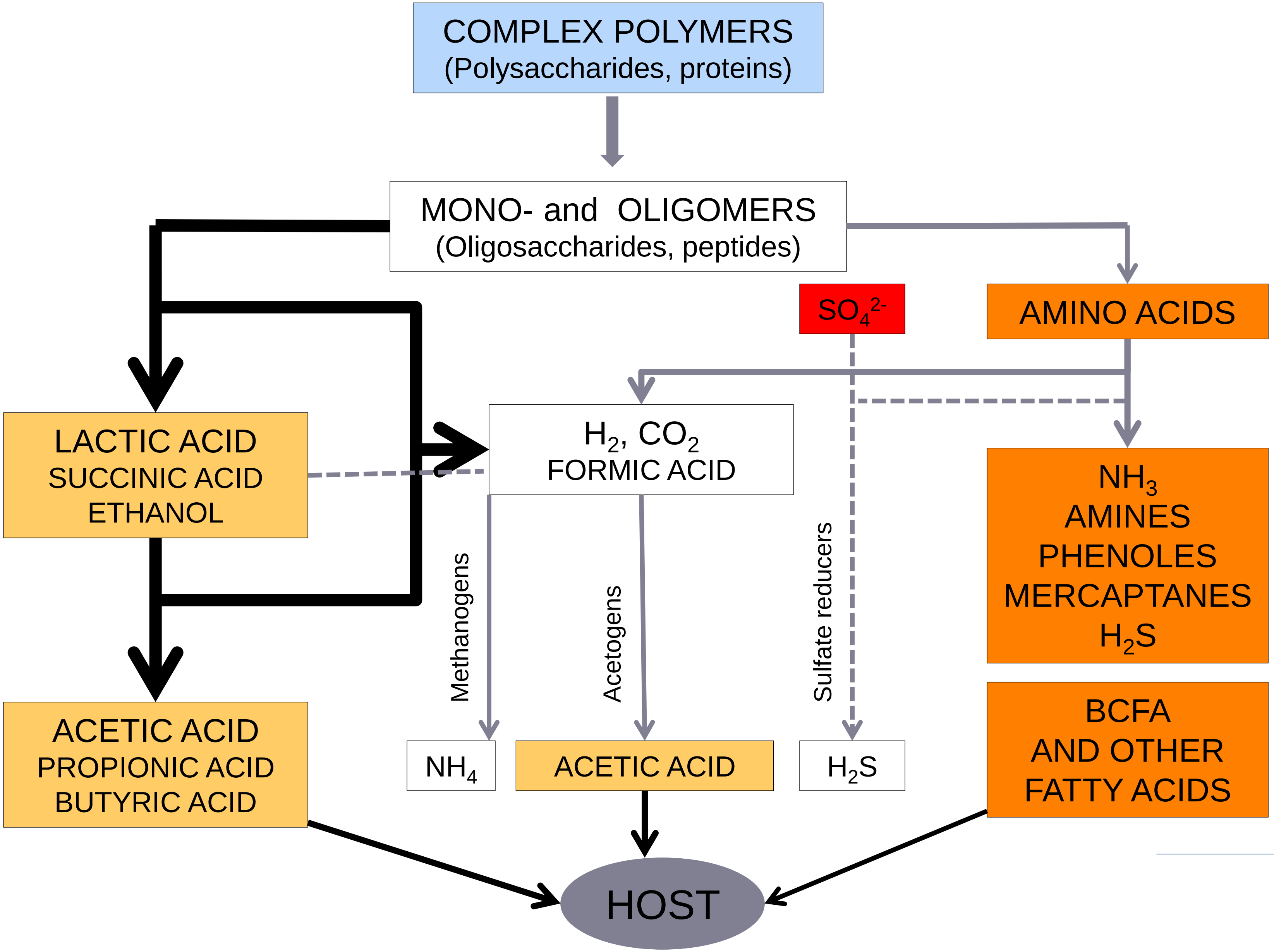
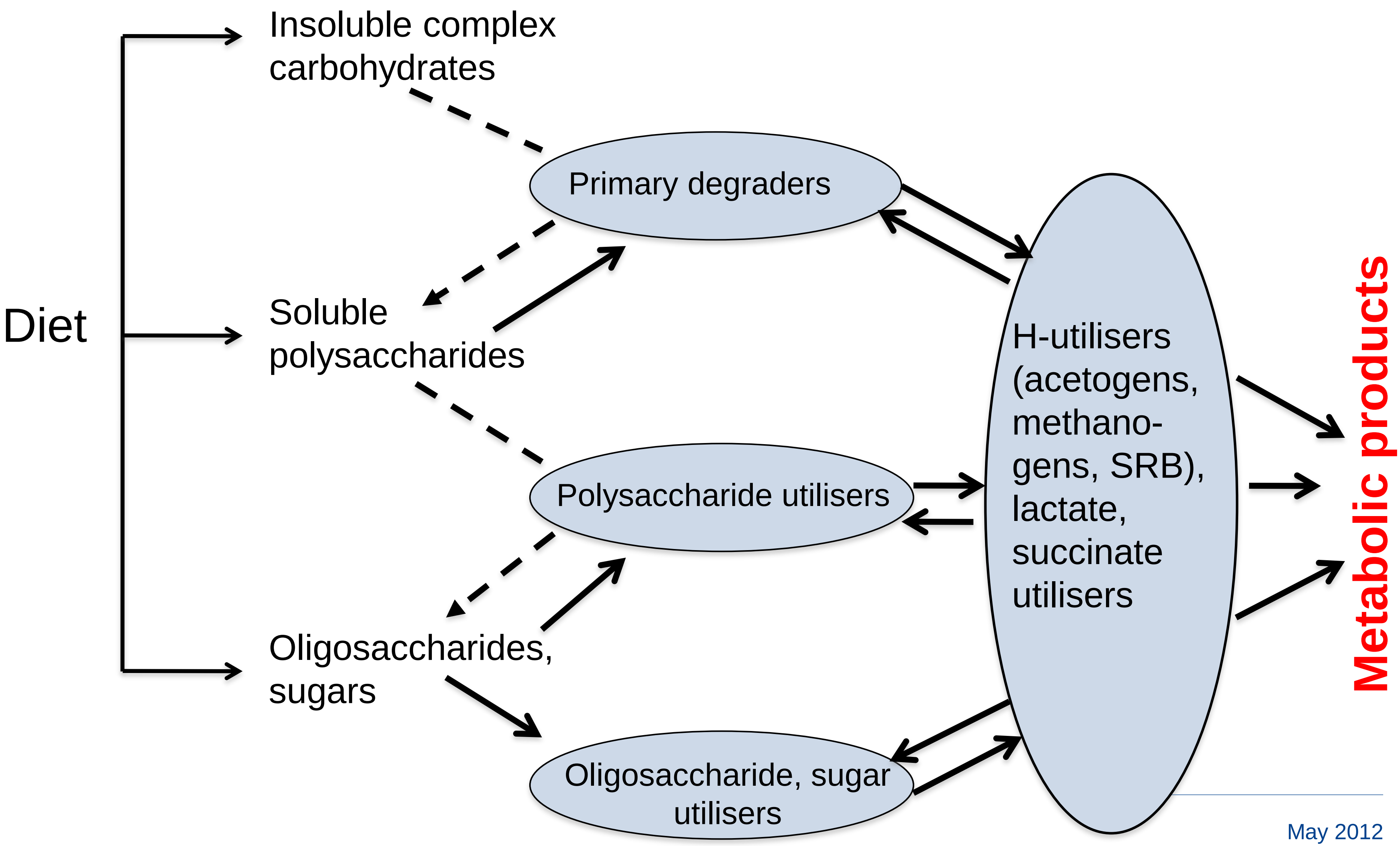
Breakdown of complex polymers by intestinal bacteria



Factors affecting butyrate formation - coss-feeding interactions



Flint et al. (2007).

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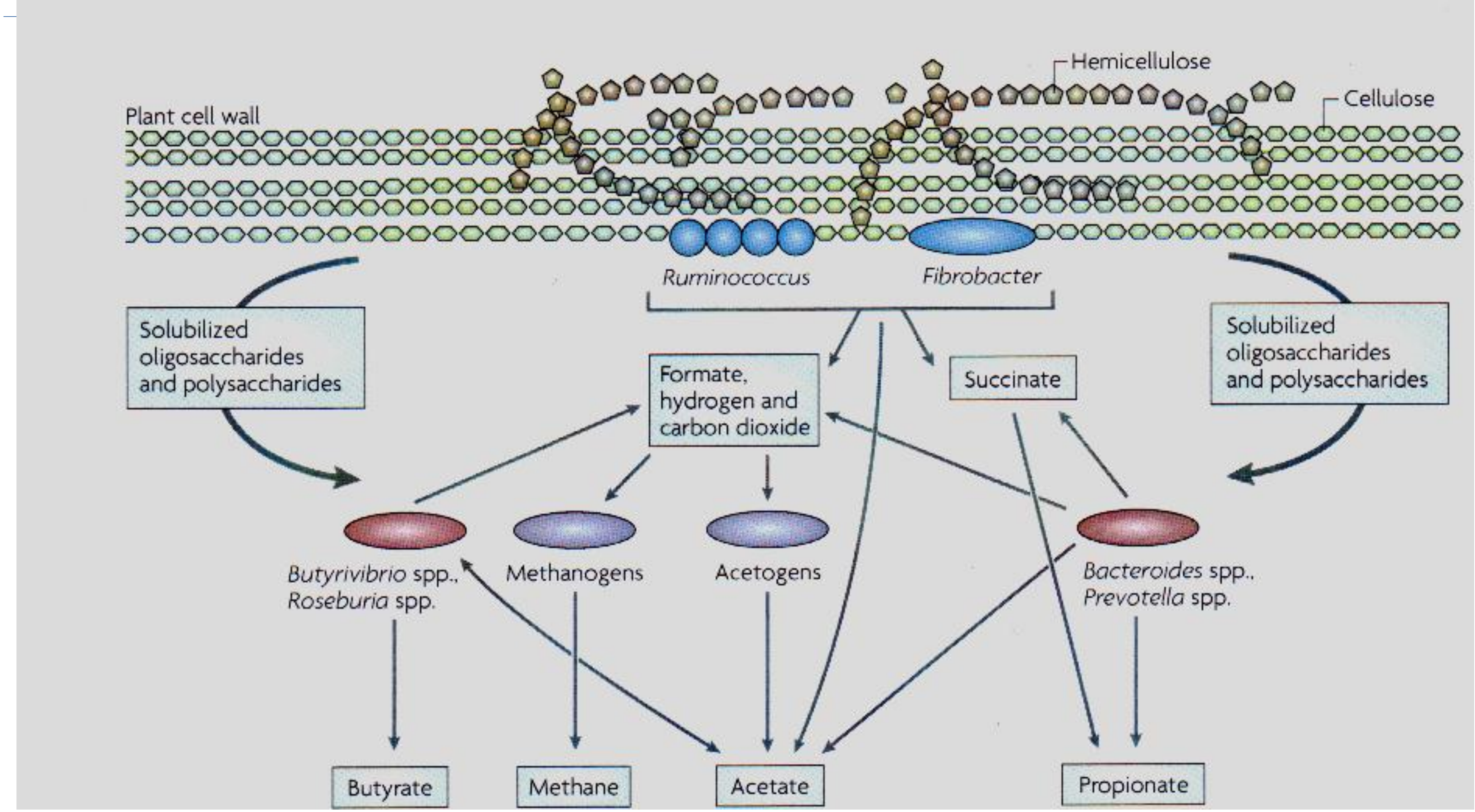
Example of metabolic cross feeding



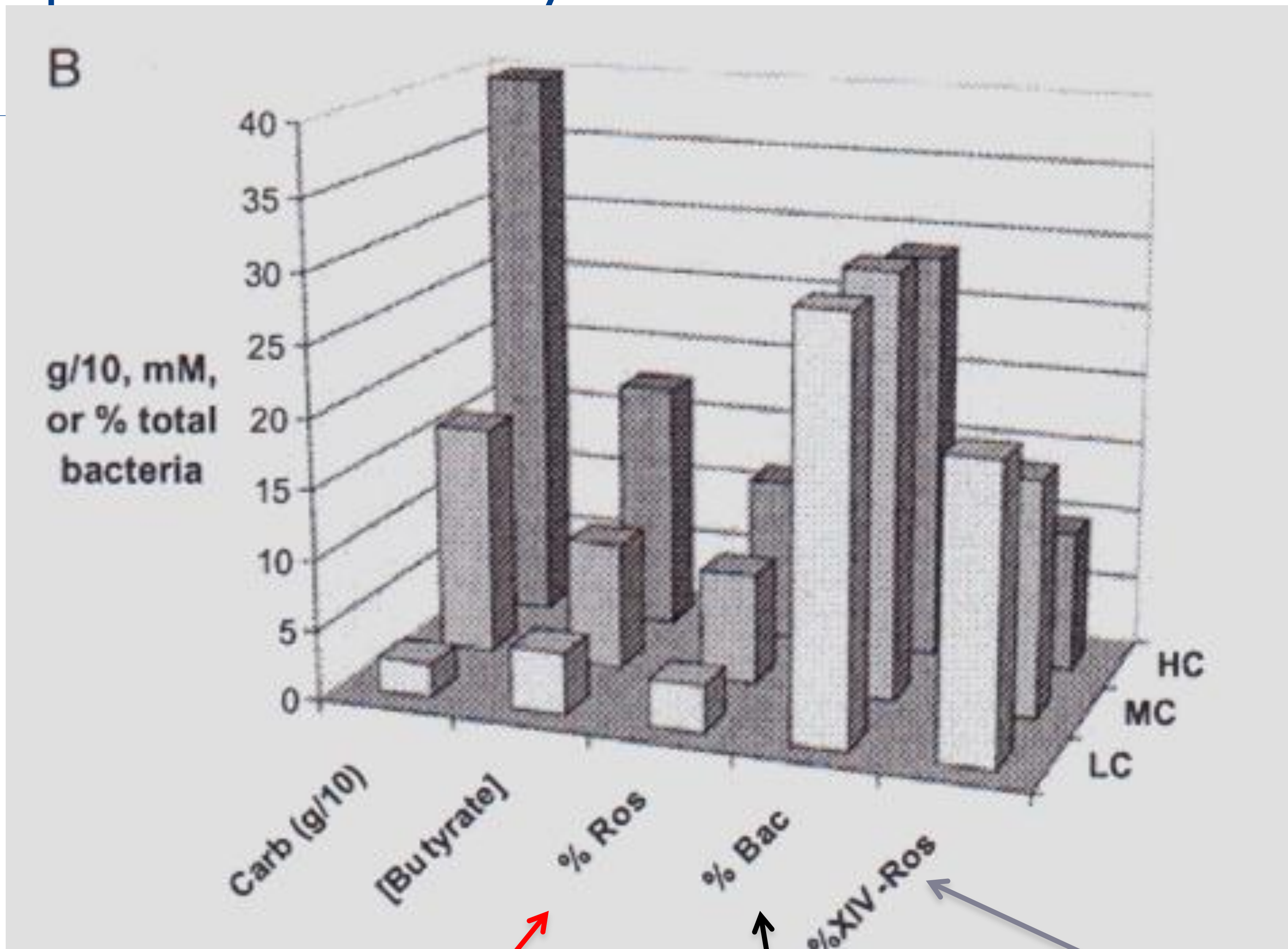
Flint et al. (2008)

Acetate – butyrate exchange

	Mixed	Amylo-pectin	Pectin	Inulin	Xylan
Concentration (mM)					
Acetate	27.2	22.3	28.7	23.7	21.7
Butyrate	4.9	5.3	3.1	3.4	4.2
Propionate	26.8	22.3	9.1	17.3	12.2
Flows (mmol C_2/d per L)					
Acetate flux	31.9	26.2	28.4	33.0	31.7
Butyrate production	10.7	10.9	5.6	5.8	9.8
From acetate	6.4	8.3	3.1	5.8	9.8
From others	4.3	2.7	2.4	0.8	0.9
Proportion from acetate	0.60	0.78	0.56	0.87	0.90

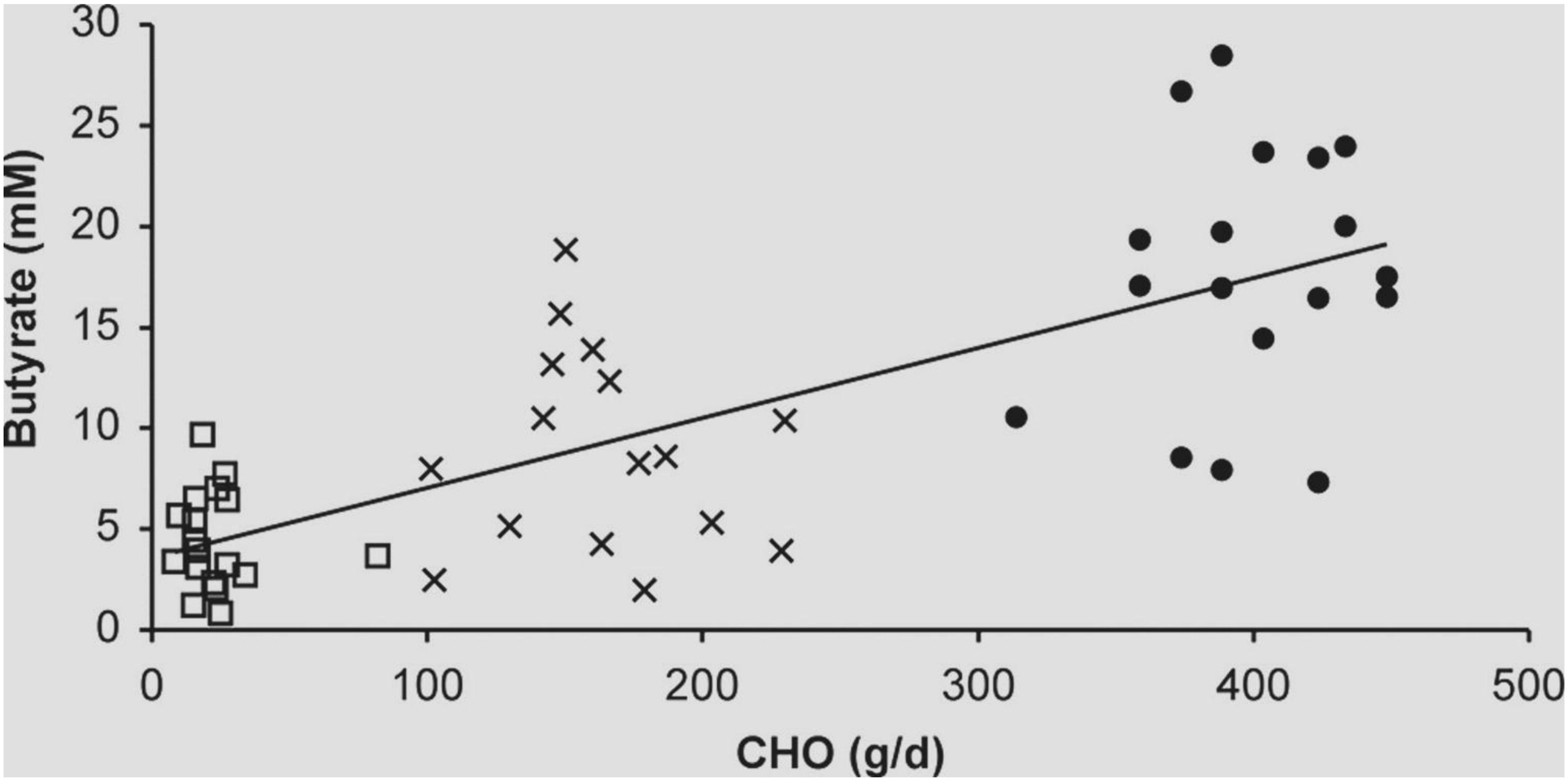
Duncan et al. (2004).

Influence of amount of carbohydrates for bacteria composition and butyrate formation



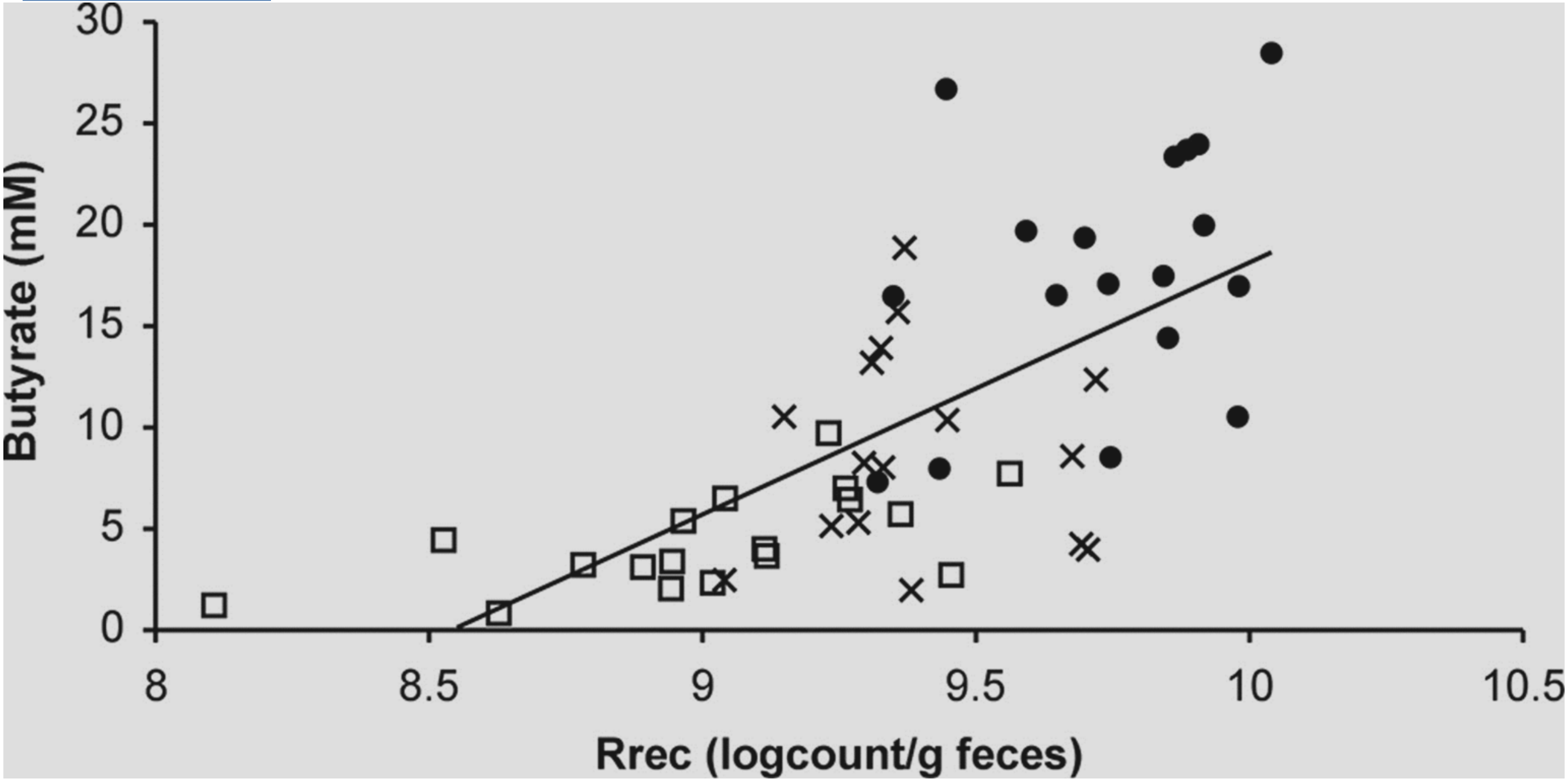
Duncan et al (2006)

Influence of carbohydrate on butyrate formation



Duncan et al. (2007)

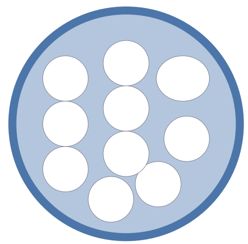
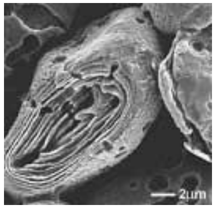
Influence of microbial composition on butyrate production

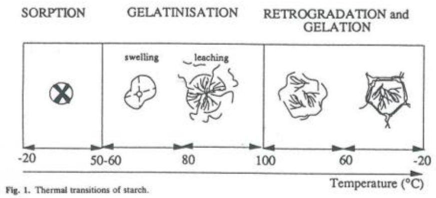


Roseburia/E. rectale group

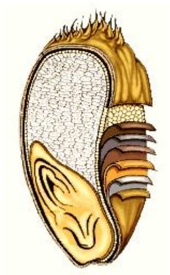
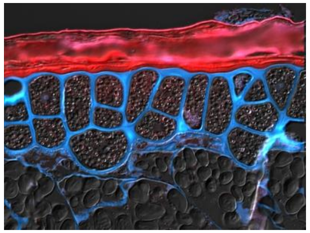
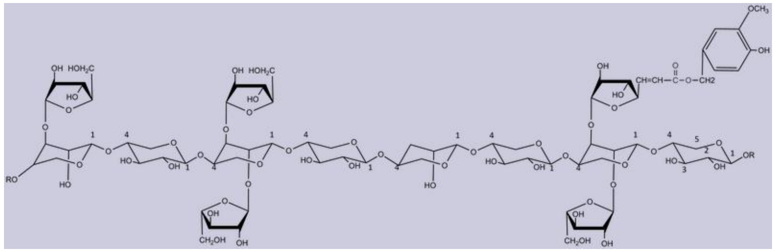
Example of butyrogenic carbohydrates and derivatives

- Resistant starch

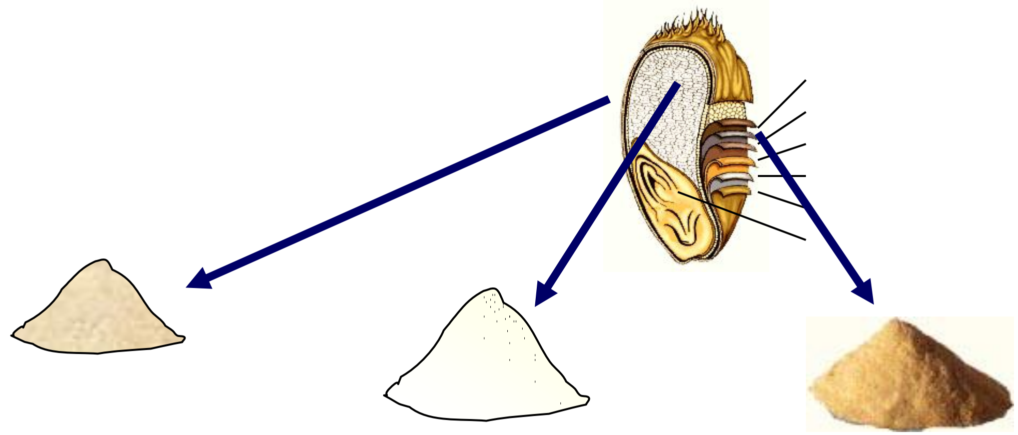


- Arabinoxylan (from oats, rye and wheat)

- Inulin
- Oligofructose
- Butyrylated starch
- Etc.

Wheat and rye fractions



Wheat fractions:

Tiger

Whole grain flour

100 % flour after peeling

100 % flour after pearling

Standard white flour

Aleurone 1

Aleurone 2

Bran

Fine bran

Fine bran <200 µ

Peeling

Pearling

Crousty

Whole grain flour

Standard white flour

Bran

Rye fractions:

Whole grain flour, ash 1.8-2 %

Flour, ash 0,65-0,75

Flour, ash 0,8-0,9 &

Rye flour, ash 3-3,5 %

Rye bran, ash 6-7 %

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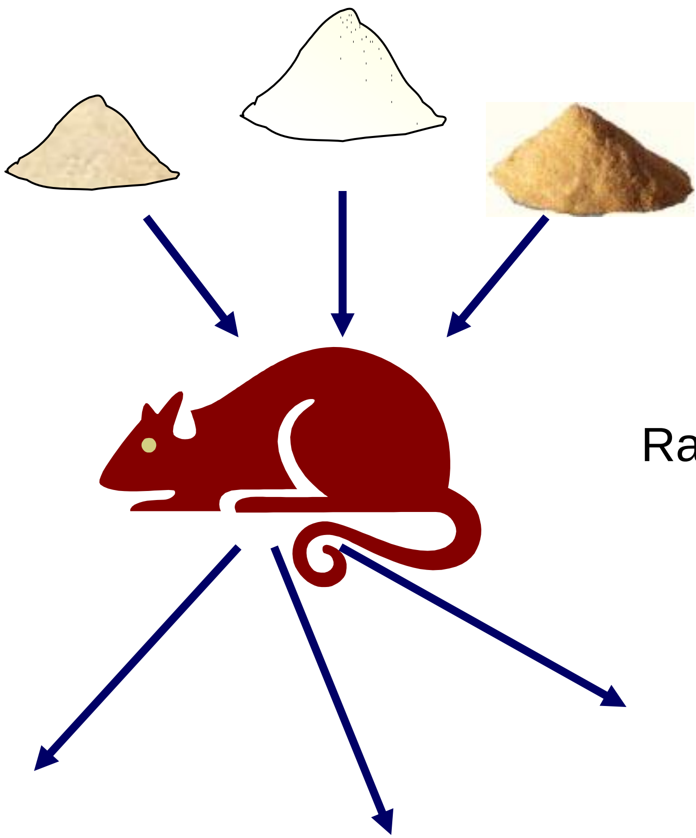
Experimental setup



Iso-DF, protein and fat diets based on wheat and rye fractions

Rat as biological filter

Blood from vena cava and the portal vein

Tissue samples

Digesta from caecum

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Diets

Table 1. Ingrediens, g/kg as is

	Mean	SD
Sugar	70	0
Cholesterol, 95%	15	0
Cholinechloride	1	0
Egg powder	51	0
Vitamin mix.	8	0
Mineral mix.	38	0
Rape seed oil	14	6
Casein	81	28
Starch	273	236
Cereal fraction	0-757	
Vitacell[1]	0-97	

[1]Vitacell ~ wheat fibre (cellulose=73%)

Table 2. Chemical composition[1], g/kg dry matter

	Mean	SD
Dry matter, %	92	2
Ash	4.5	0.4
Protein	161	9
Fat	71	3
Carbohydrates		
Sugars	63	6
Glucose	2	1
Fructose	1	0
Sucrose	60	5
Fructan[2]	4	4
Starch	542	20
Non-starch polysaccharides (NSP)	82	7
Klason lignin	15	5
Dietary fibre (NSP + lignin)	97	9

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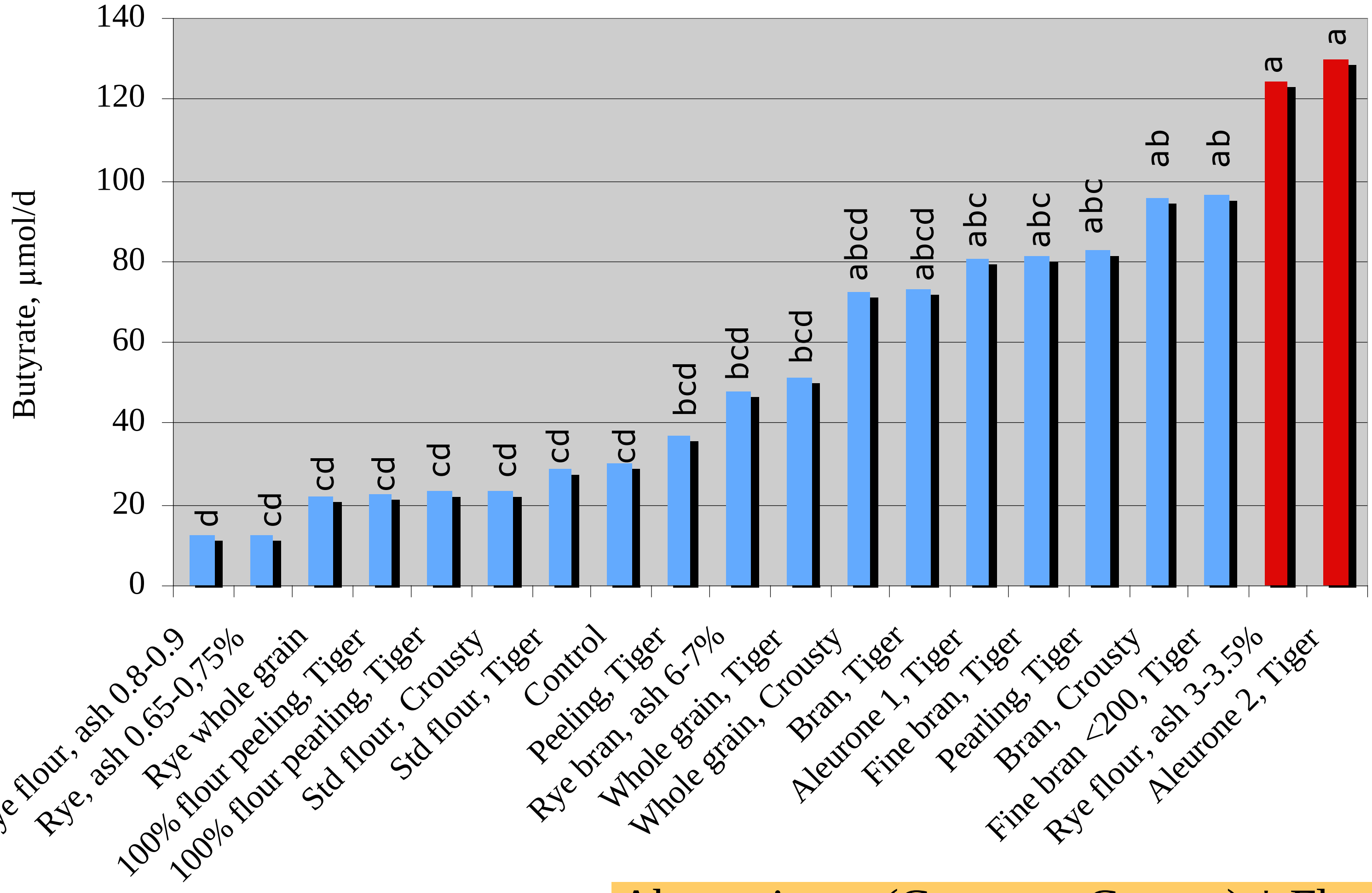
Butyrate ”absorption”



Absorption = ($C_{portal\ vein}$ - $C_{vena\ cava}$) * Flow

Flow[1] (ml/min) = 0.297 * X + 0.344

X = weight of caecal content (g)

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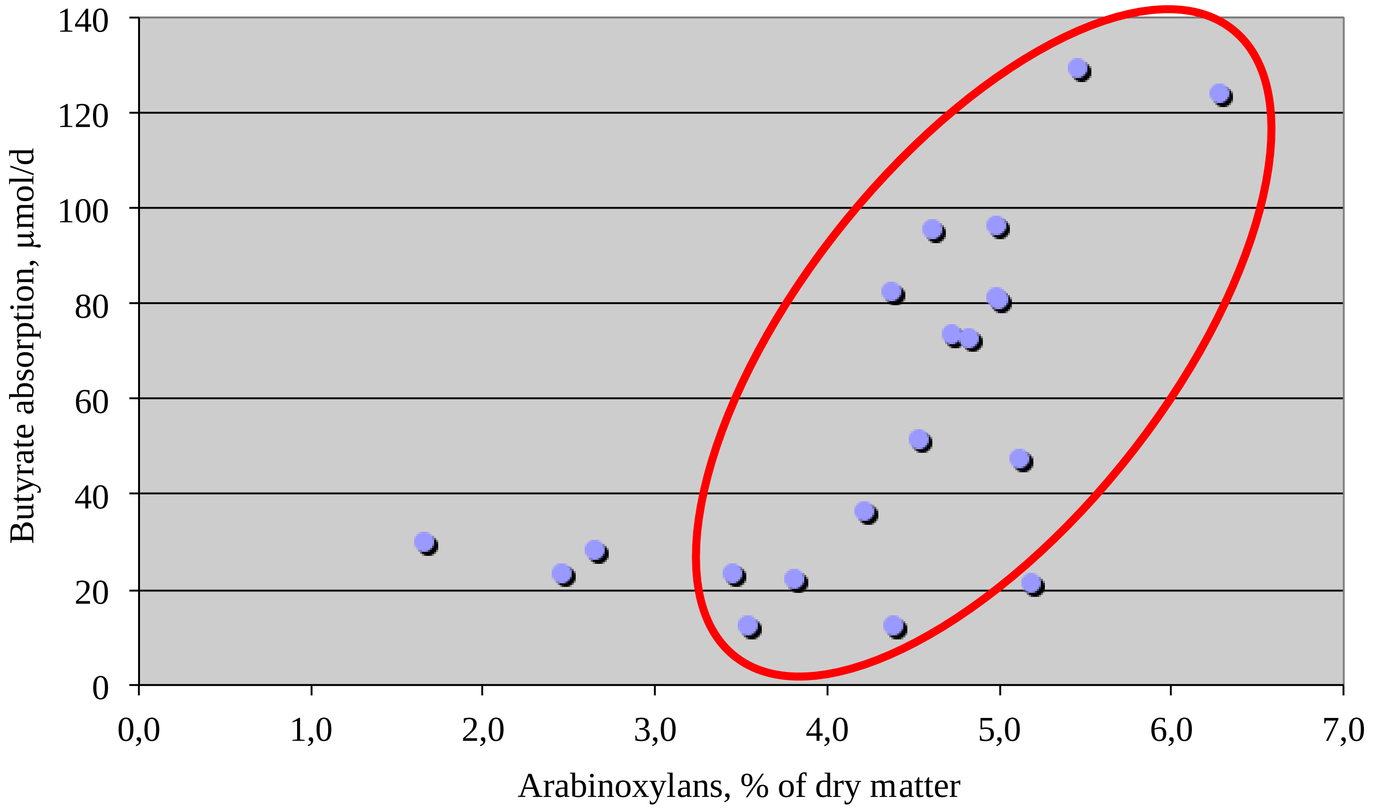
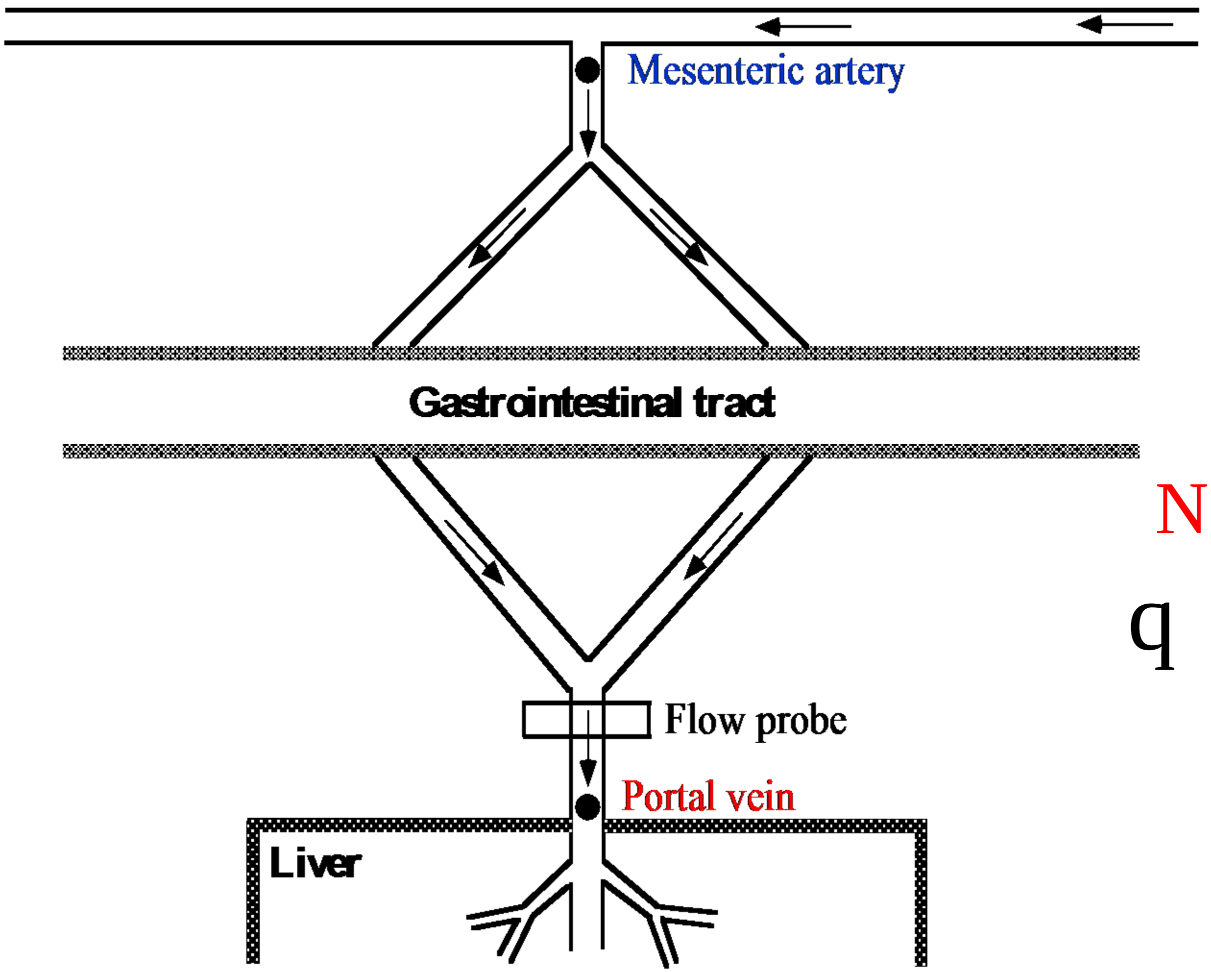
Correlation between arabinoxylan and butyrate ”absorption”



The catheterised pig model



Net absorption

$$q = (C_p - C_a)\, F\, (dt)$$

$$Q = \sum_{t_0}^{t_n} q$$

Production of SCFA and butyrate and molar proportion of butyrate in absorbed SCFA

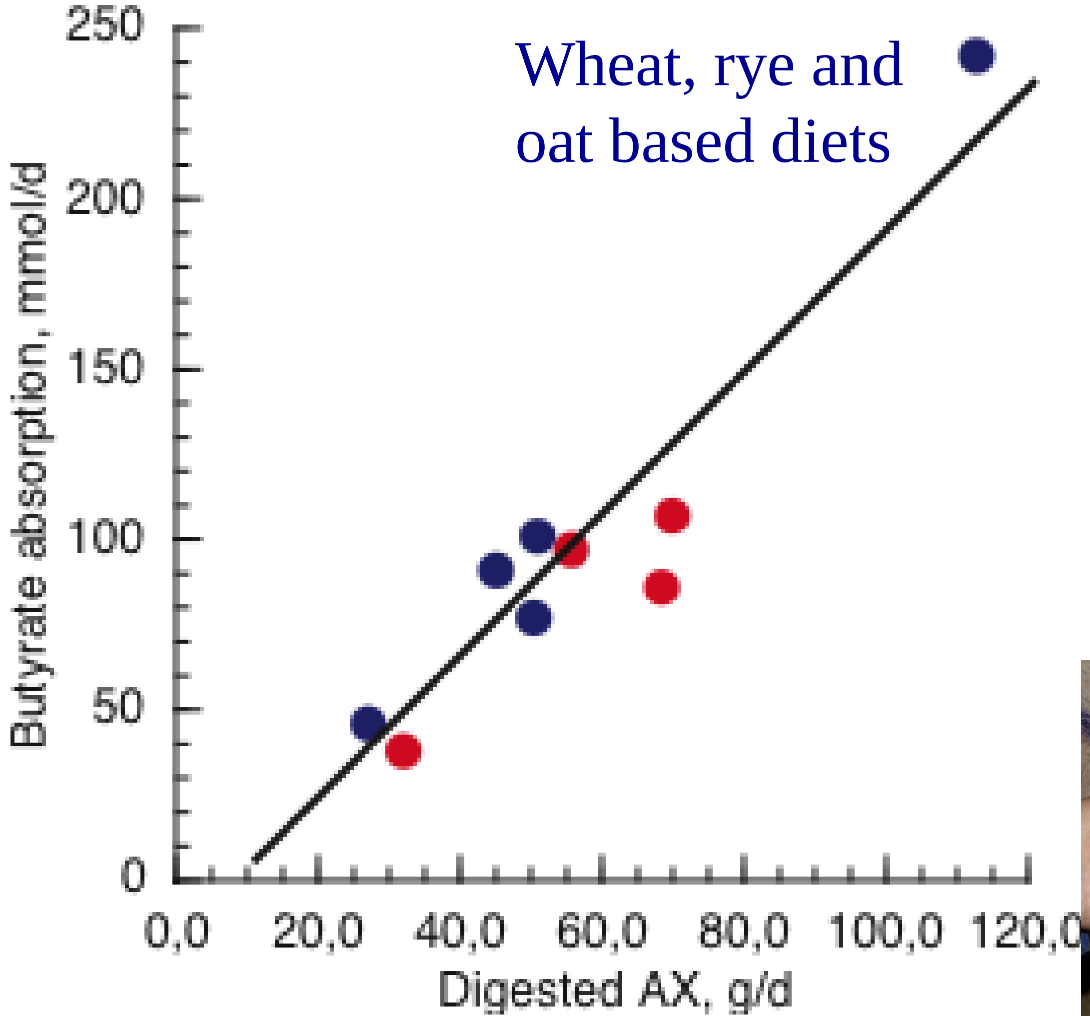
Diet type	mmol/h T-SCFA	mmol/h Butyrate	Butyrate %
Low fibre wheat	30	1.9	6.4
+ wheat bran	31	3.2	10.4
+ oat bran	36	4.1	11.3
Wheat flour + Vitacel	34	1.6	4.9
Whole wheat grain	36	3.6	9.6
Rye aleurone rich flour	41	4.4	10.9
Wheat aleurone	40	4.0	9.9
Wheat bread (high fibre)	66	3.8	5.7
Whole grain rye (high fibre)	77	10.1	13.1
Corn	20	1.3	6.5
Corn/potato	60	15.8	26.3
Potato	89	23.8	26.7

AX effect

RS effect

van der Meulen et al. (1997); Bach Knudsen et al (2001; 2005; unpublished).

Relationship between AX degradation and butyrate absorption in different cereal diets



Bach Knudsen & Lærke (2010).

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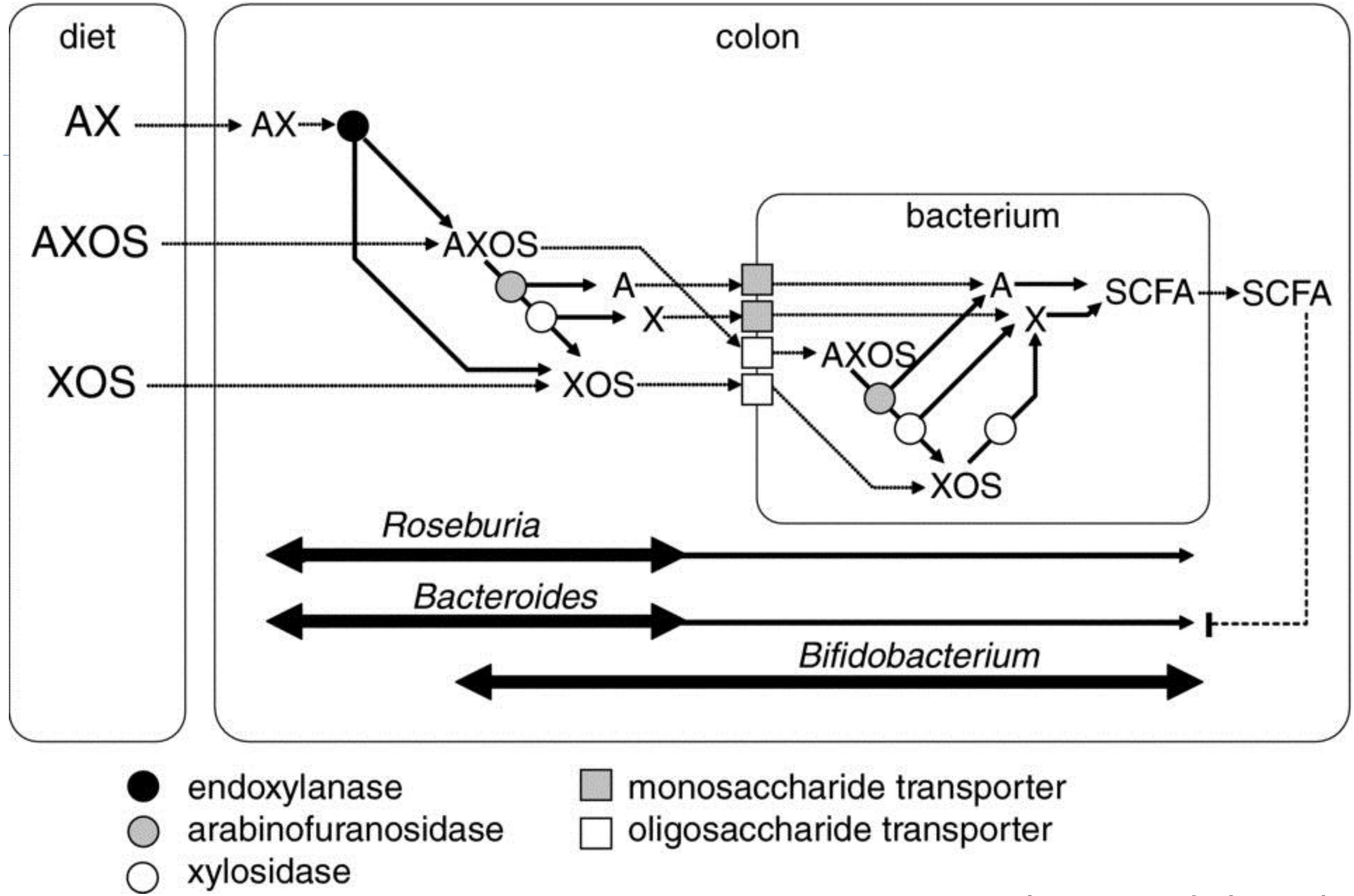
Proposed fermentation of AX, AXOS and XOS

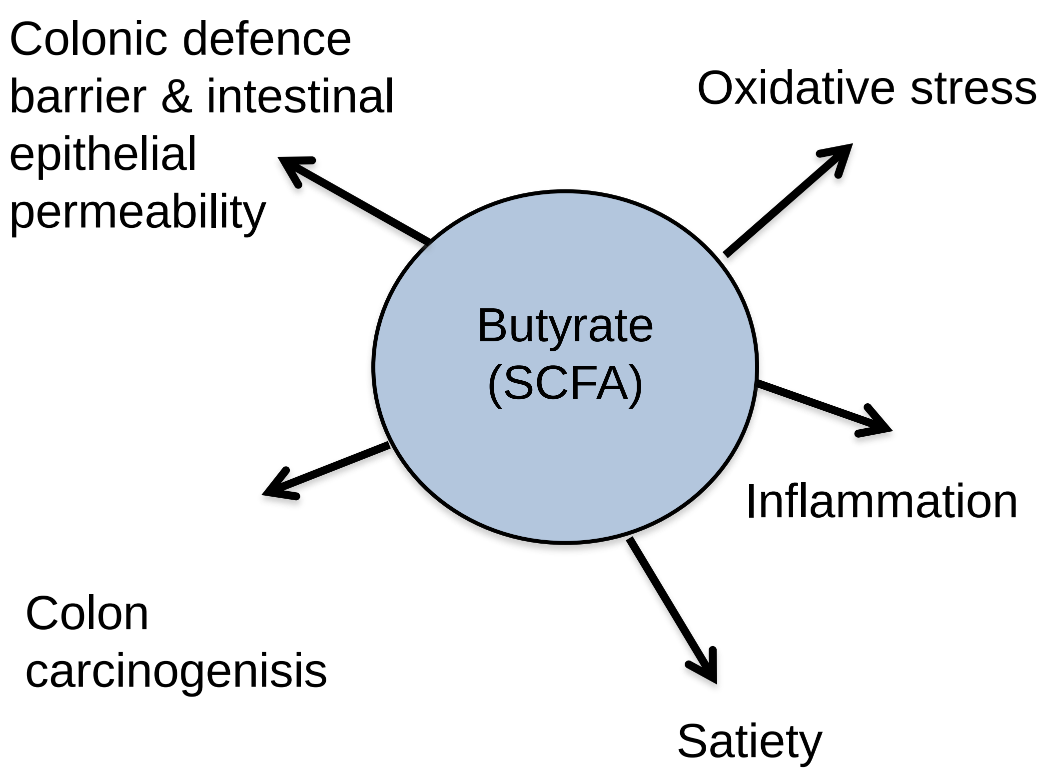


Broekaert et al. (2012)

Functions of butyrate in the gut

- Butyrate along with other SCFA's is produced by microbial fermentation in the large intestine
- The main substrates for microbial production of butyrate are the dietary residues not digested in the small intestine

Different domains that may be affected by butyrate produced in the colon



Hamer et al. (2008).

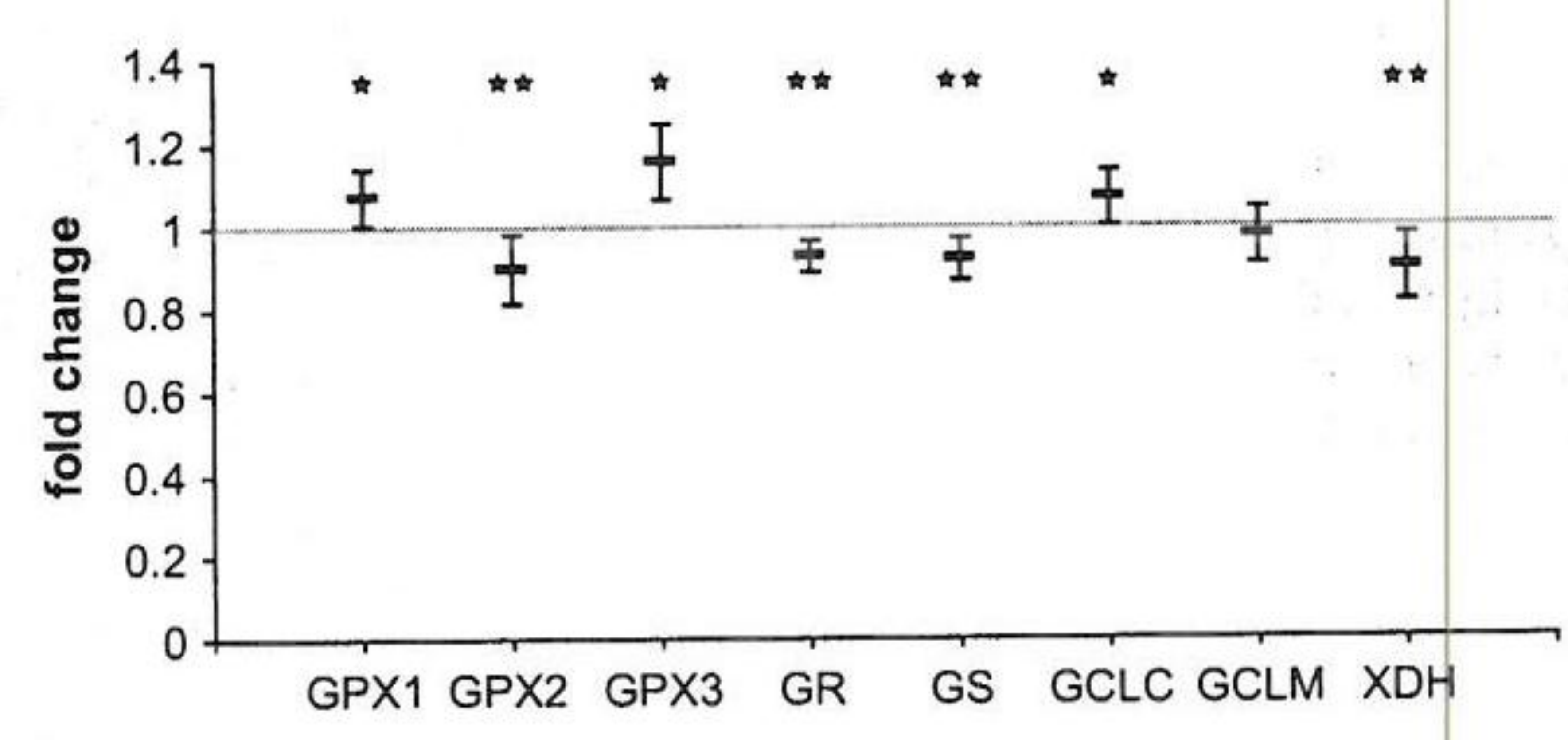
Effect of butyrate enemas (100 mM) on parameters related to oxidative stress

	Placebo	Butyrate	*p*-Value
	n = 16	*n* = 16	
TEAC (nmol trolox Eq/mg protein)	137.2 (112.6–210.7)	136.2 (116.2–207.6)	0.72
Uric acid (nmol/mg protein)	3.1 (1.9–3.3)	2.4 (1.6–3.7)	0.01
GST (U/mg protein)	0.34 (0.23–0.51)	0.32 (0.24–0.41)	0.55
tGSH (nmol/mg protein)	24.5 (13.4–32.4)	27.2 (21.9–35.5)	0.03
GSH (nmol/mg protein)	22.9 (12.0–31.5)	26.5 (20.4–35.2)	0.03
GSSG (nmol/mg protein)	0.4 (1.9–2.13)	0.31 (0.08–2.69)	0.18
GSH/GSSG ratio	71.1 (8.1–127)	90.8 (7.6–333.9)	0.07
MDA (nmol/mg protein)	1.09 (0.69–2.2)	0.78 (0.44–1.44)	0.13

Values expressed as median (range).
TEAC, trolox equivalent antioxidant capacity; GST, glutathione-*S*-transferase; tGSH, total glutathione; GSH, reduced glutathione; GSSG, glutathione disulfide; MDA, malondialdehyde.

Hamar et al. (2009).

Effects of butyrate on oxidative markers in mucosa



Hamar et al. (2009).

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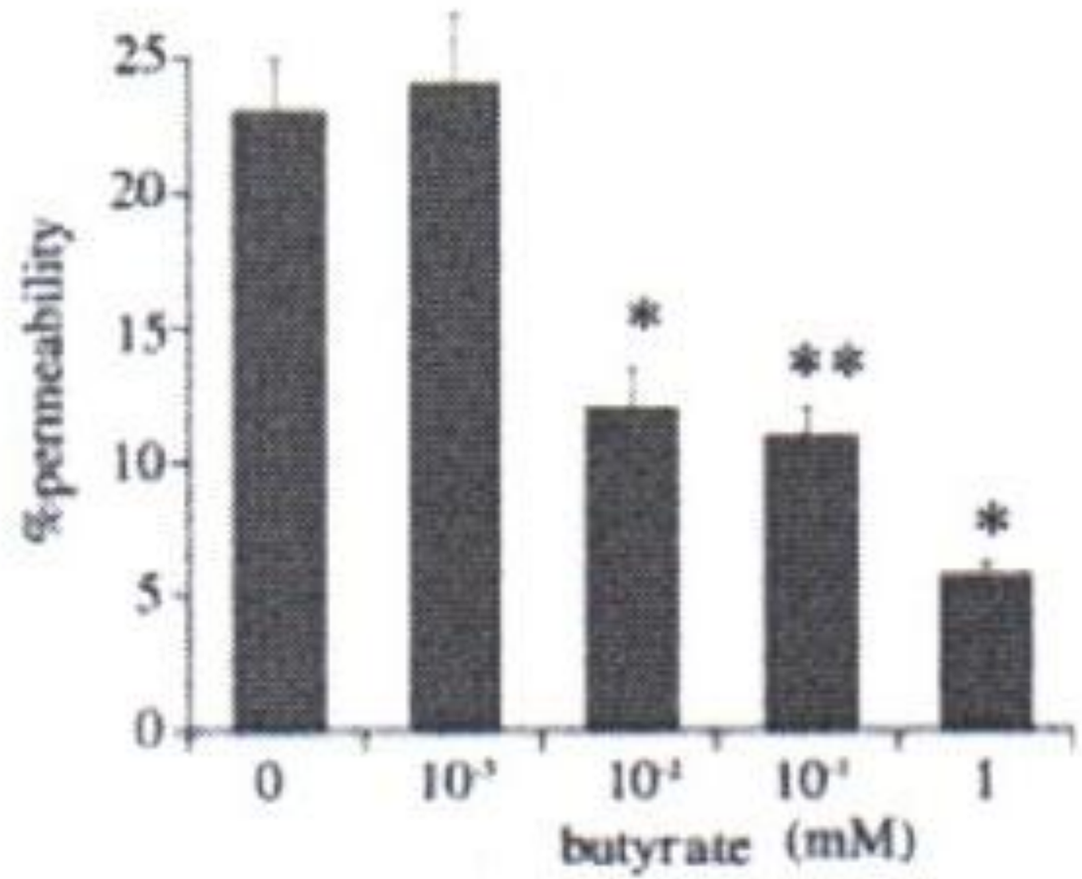
Influence of butyrate on permeability in vitro



Concepts for enhanced colonic production of butyrate and influence on colonic health and insulin sensitivity - ButCoIns

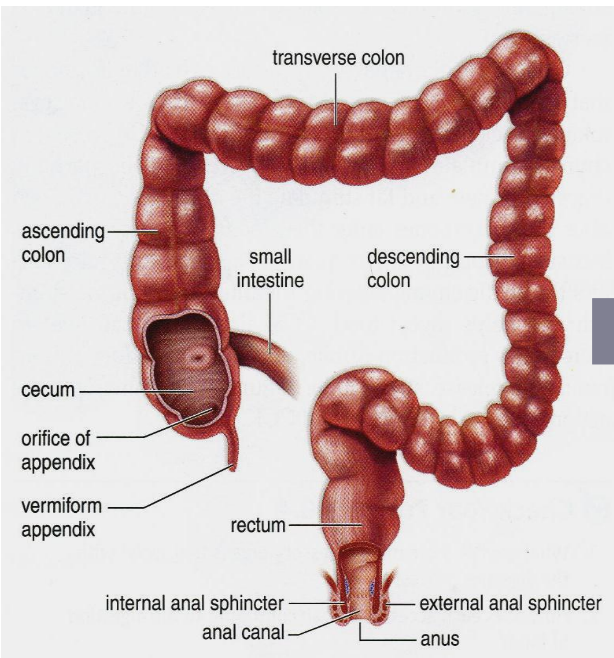
- Aarhus University, Department of Animal Science, PI Prof Knud Erik Bach Knudsen
- Aarhus University Hospital, Department of Gastroenterology and Hepatology
- Aarhus University Hospital, Department of Endocrinology and Metabolism
- University of California, Davis
- Companies:
  - Dupont (formerly Danisco)
  - Lantmännen Foods
  - KMC

Objective and aims

- The **overall objective** is to improve colonic health, peripheral insulin sensitivity and glucose homeostasis by increased colonic butyrate production brought about by pre-, pro- and synbiotic concepts
- Specific aims:
  - **Quantify** the implication of pre- and probiotics on colonic butyrate production
  - **Develop** novel synbiotic concepts for improved butyrate production
  - **Document** the impact of enhanced butyrate production on colonic health parameters
  - **Document** the impact of increased butyrate production on insulin sensitivity and glucose homeostasis

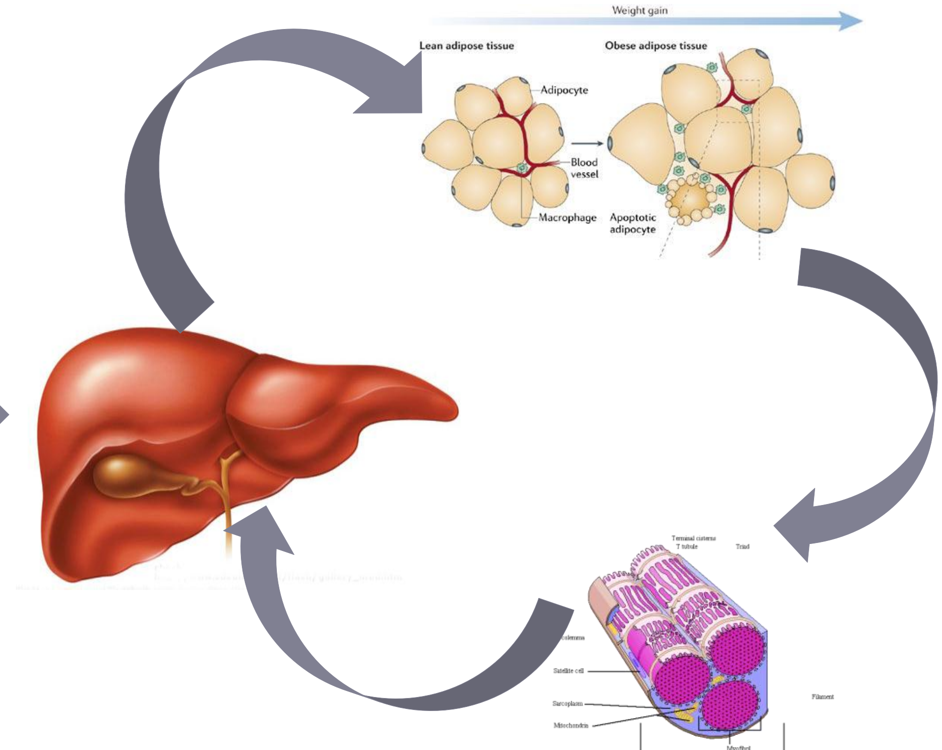
WP1: Prebiotics and diets

WP2: Gut health



Oxidative stress of colonic mucosa
Maintained gut barrier function
Inflammatory response
Microbial composition

WP3: Insulin sensivity



Improved whole body insulin sensitivity
Altered metabolism in skeletal muscle and adipose tissue

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A carbohydrate component that unite projects

Enz mod. starch

β-glucan

Xylanases 1
(GUDP)

Xylanases 2
(Industrial PhD)

Arabinoxylan

BioFunCarb
(DSF)

ButCoIns
(DSF)

RS

Key words: Feed efficiency, environmental impact

Key words: GI, gut health, insulin sensitivity, metabolic syndrome

ANIMAL nutrition

HUMAN nutrition

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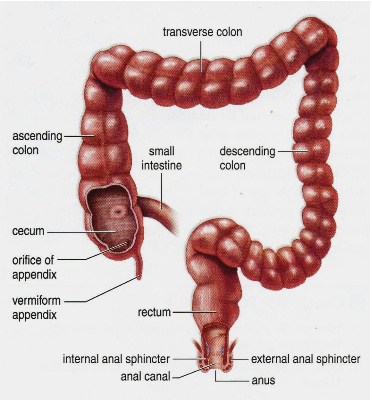
Summary

- Butyrogenic carbohydrates represent a subfraction of prebiotics that has the ability to stimulate the production of butyrate
- It is produced by selected groups of microorganisms in the large intestine either directly or by cross-feeding
  - Resistant starch
  - Arabinoxylan
- Important nutrient for the intestinal epithelial
  - Permeability
  - Oxidative stress
  - Colon carcinogenisis
  - Inflammation



Thank you very much for your attention