UM | Faculty of Agricultural and Food Sciences

Enhancing gut health of dairy cows

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Gut health

- Healthy digesta
  - Healthy chemical composition: pH, osmolality, redox potential, nutrients, low toxins
  - Healthy microbiota: high abundances and functionality of beneficial microbes, low abundances and functionality of pathogenic microbes
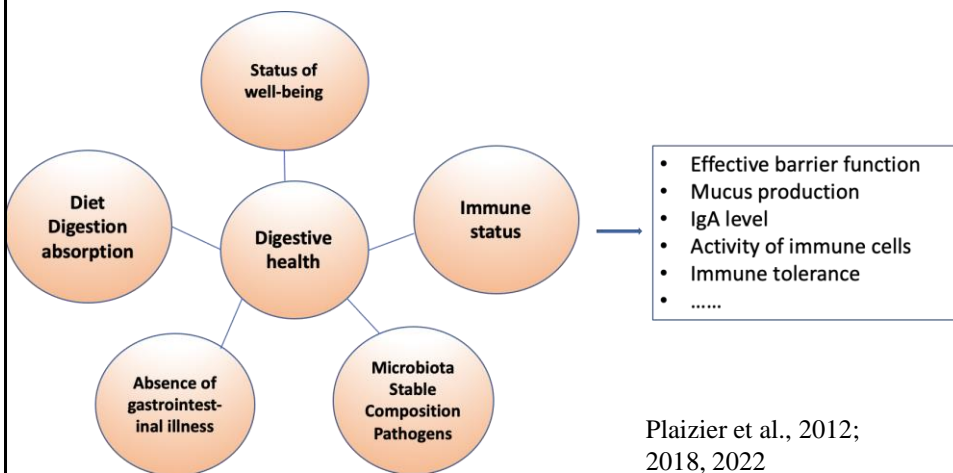 - Healthy physical composition:
    - Structure

Gut health

- Healthy mucosa/epithelium
  - Absorption of nutrients
  - Barrier function
  - Immune response
- Motility

Factors affecting gut health

- Status of well-being
- Diet Digestion absorption
- Digestive health
- Immune status
- Absence of gastrointestinal illness
- Microbiota Stable Composition Pathogens

- Effective barrier function
- Mucus production
- IgA level
- Activity of immune cells
- Immune tolerance
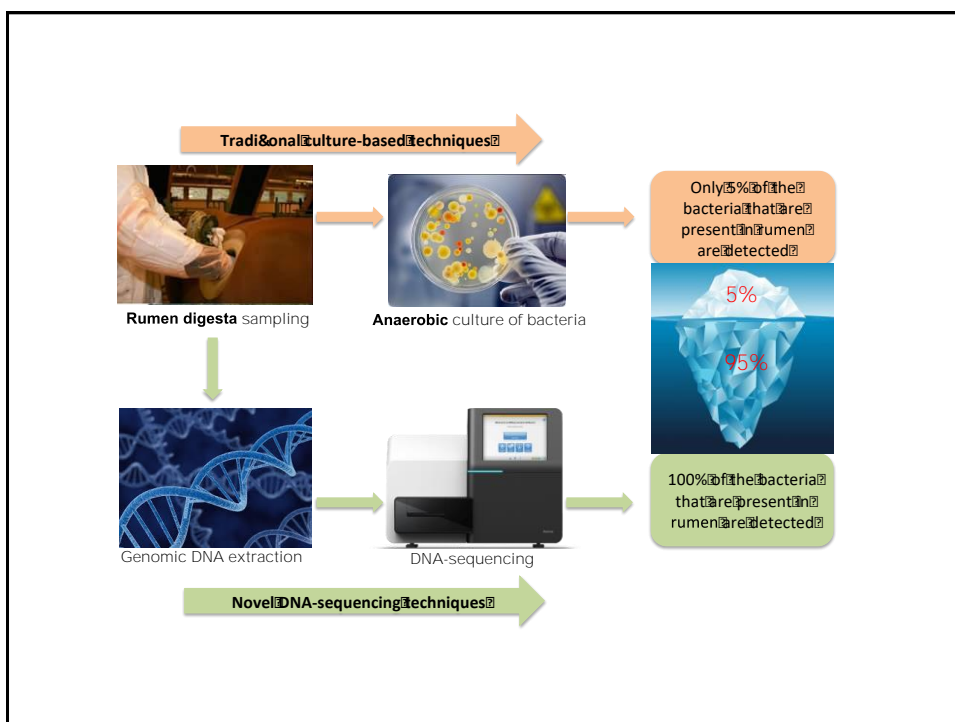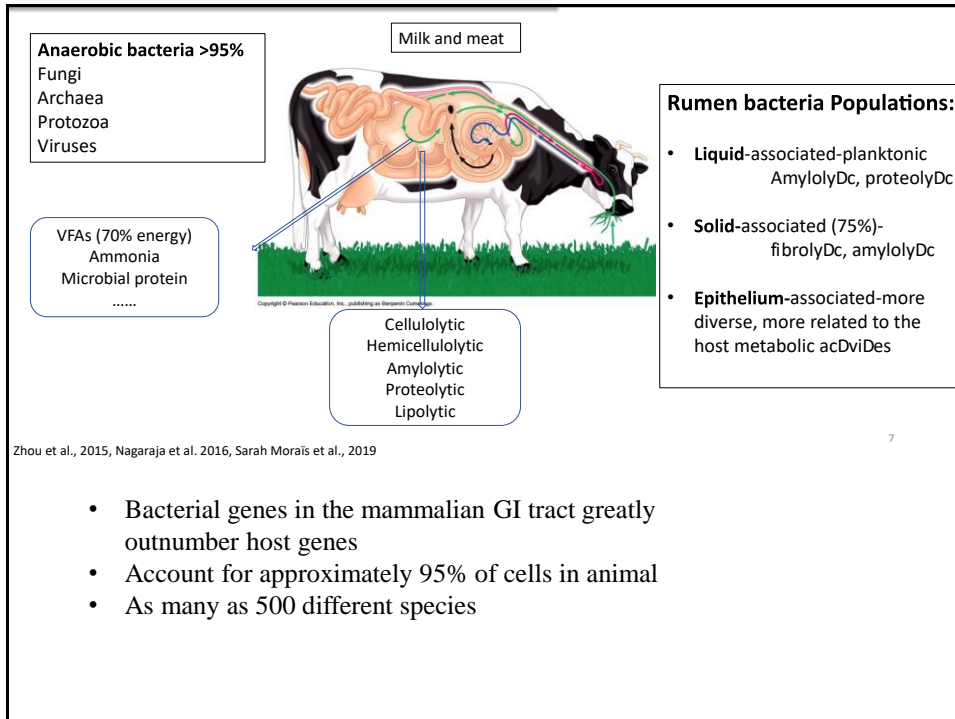- ......

Plaizier et al., 2012; 2018, 2022

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2

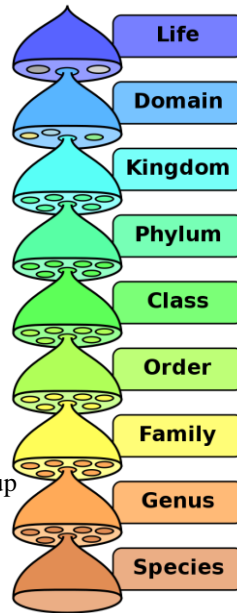## Causes of SARA on farms (Plaizier et al., 2018, 2022):

- Excess grain/starch in diet
- Ingested diet different from formulated diet.
  - Mixing errors and sorting against coarse feed particles
- Insufficient coarse fiber/rumination/saliva
- Finely chopped silage and excessive mixing
- Low buffering capacity forages
  - High protein forages (e.g. alfalfa/luzerne) more buffering capacity than low protein forages (e.g. corn/maize silage)
- Very digestible forages/pasture
  - Low NDF content and high moisture and sugar contents

Causes of SARA on farms (Plaizier et al., 2018, 2022):

- Insufficient absorption of VFA
  - Papillae take time to adapt to increased VFA production.
- Large meals rather than smaller meals
  - Empty feed bunk, competition at feed bunk
- Heat stress
- Susceptible cows/microbiota
- Combination of these and other factors
- Excessive grain feeding good model for SARA induction?
  - "Grain induced" SARA more severe than " forage induced SARA"

Slide 1

Anaerobic bacteria >95%
Fungi
Archaea
Protozoa
Viruses

Milk and meat

VFAs (70% energy)
Ammonia
Microbial protein
......

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Cellulolytic
Hemicellulolytic
Amylolytic
Proteolytic
Lipolytic

Rumen bacteria Populations:

- **Liquid**-associated-planktonic
  AmylolyDc, proteolyDc

- **Solid-**associated (75%)-
  fibrolyDc, amylolyDc

- **Epithelium-**associated-more
  diverse, more related to the
  host metabolic acDviDes

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Zhou et al., 2015, Nagaraja et al. 2016, Sarah Moraïs et al., 2019

- Bacterial genes in the mammalian GI tract greatly outnumber host genes
- Account for approximately 95% of cells in animal
- As many as 500 different species

Slide 2

TradiDonal culture-based techniques

Rumen digesta sampling

Anaerobic culture of bacteria

Only 5% of the bacteria that are present in rumen are detected

5%

95%

Genomic DNA extraction

DNA-sequencing

100% of the bacteria that are present in rumen are detected

Novel DNA-sequencing techniques

Taxonomy

Operational Taxonomic Unit (OTU) : group of bacteria with a 16S marker gene sequence identity of 97 %.
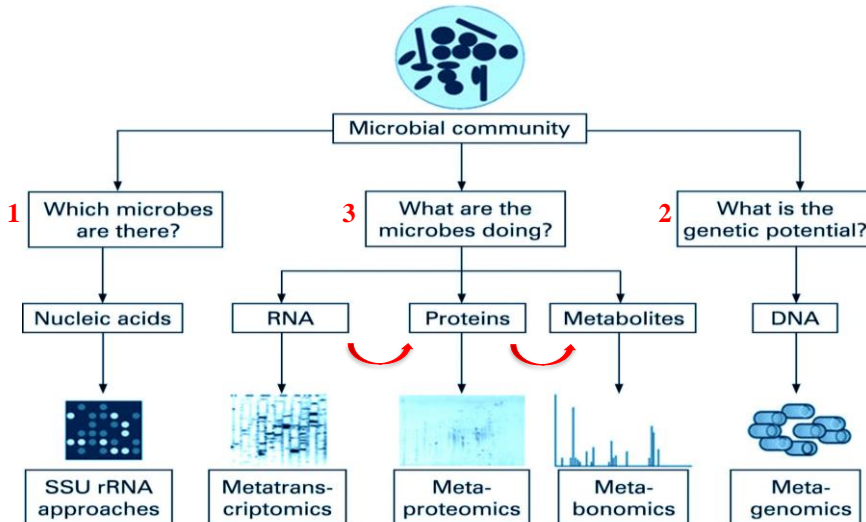New: Amplicon Sequence Variant (ASV)

Life
Domain
Kingdom
Phylum
Class
Order
Family
Genus
Species

Table 1. The characteristics of predominant ruminal bacteria. Abbreviations are as follows: CU, cellulose; HC, hemicellulose; DX, dextrins; SU, sugars; ST, starch; PC, pectin; XY, xylans; L, lactate; S, succinate; GL, glycerol; AA, amino acids; OA, organic acids; H_2, hydrogen; F, formate; CO_2, carbon dioxide; A, acetate; E, ethanol; B, butyrate; L, lactate; P, propionate; Br, branched-chain volatile fatty acids; and CH_4, methane.

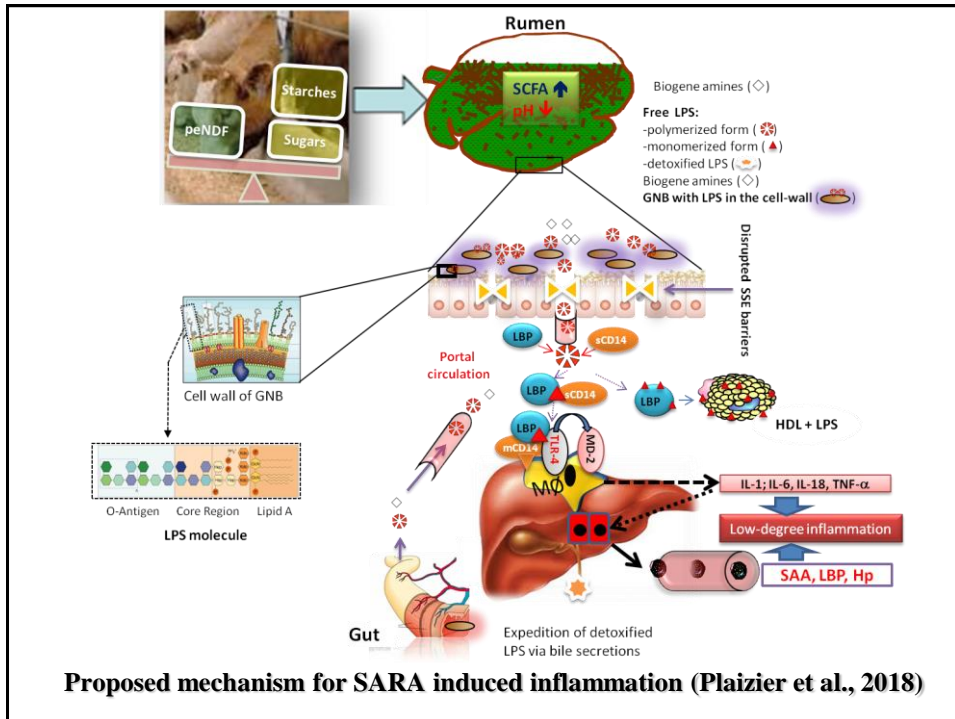| Species	Ruminal niche	Fermentation products
Fibrobacter succinogenes	CU	S, F, A
Ruminococcus albus	CU, HC	A, F, E, H_2
Ruminococcus flavefaciens	CU, HC	S, F, A, H_2
Eubacterium ruminantium	HC, DX, SU	A, F, B, L
Ruminobacter amylophilus	ST	S, F, A, E
Streptococcus bovis	ST, SU	L, A, F, E
Succinomonas amylolytica	ST	S, A, P
Prevotella ruminocola, albensis, brevis, and bryantii	ST, PC, XY, SU	S, A, F, P
Butyrivibrio fibrisolvens	ST, CU, HC, PC, SU	B, F, A, H_2
Selenomonas ruminantium	ST, DX, SU, L, S	L, A, P, B, F, H_2
Megasphaera elsdenii	L, SU	P, A, B, Br, H_2
Lachnospira multiparus	PC, SU	L, A, F, H_2
Succinivibrio dextrinosolvens	PC, DX, SU	S, A, F, L
Anaerovibrio lipolytica	GL, SU	A, S, P
Peptostreptococcus anaerobius	AA	Br, A
Clostridium aminophilum	AA	A, B
Clostridium sticklandii	AA	A, Br, B, P
Wolinella succinogenes	OA, H_2, F	S
Methanobrevibacter ruminantium	H_2, CO_2, F	CH_4

Microbiota

- Richness
  - Total number of bacterial species (OTU/ASV)
- Diversity
  - α-diversity: within group diversity
    - Measure of how many different species and how evenly distributed they are in the group.
    - Combines richness with the size (evenness) of populations
  - β-diversity: diversity among groups
  - Low α- diversity: more efficient or less robust? (Ben et al., 2016; Elolimy et al., 2000)

Microbial ecology: the study of microbes in the environment and their interactions with each other and their environment (Barton et al. 2011).

Adopted from: http://www.metu.edu.tr/~bicgen/research/envt.html

Proposed mechanism for SARA induced inflammation (Plaizier et al., 2018)

Possible inaccuracies of proposed mechanism for SARA induced inflammation

- Reduced barrier function of epithelia allows translocation of immunogenic compounds other than LPS and bioamines
- Endotoxin tolerance
- How toxic is LPS from common rumen bacteria?

Effects of grain based SARA challenge (GBSC) and alfalfa pellet SARA challenge (APSC) on rumen pH, LPS, and acute phase proteins in blood of dairy cows

| Item	Control	APSC	GBSC
Time < rumen pH 5.6, h/d	56[b]	255[a]	299[a]
Cecum pH	7.07[a]	6.86[b]	6.79[b]
Starch in hindgut, % of DM	2.8[b]	2.6[b]	7.4[a]
Rumen LPS, EU/mL	8,333[c]	18,425[b]	124,566[a]
Cecal LPS, EU/mL	18,289[b]	15,631[b]	128,566[a]
Fecal LPS, EU/mL	13,909[b]	18,998[b]	101,555[a]
SAA, mg/L	38.1[b]	35.5[b]	62.1[a]
Hp, mg/L	478[b]	643[ab]	864[a]
LBP, mg/L	8.4[b]	9.3[b]	13.0[a]

Li et al., 2010

α-diversity in rumen fluid, and cecum digesta under control, alfalfa-pellet SARA challenge (APSC) or a grain-based SARA challenge (GBSC)

Sample type and treatment	Number of OTU (97% distance)	Fisher[2]	Richness indices		Diversity indices		Effective number of species
			Chao1	ACE	Shannon	Simpson	Simpson's reciprocal
Rumen fluid							
Control	1031[aA]	854[aA]	2540[A]	2725[A]	6.41[a]	0.99	302[A]
APSC	714[abB]	450[abB]	1514[AB]	1630[AB]	5.86[ab]	0.99	136[AB]
GBSC	618[bC]	338[bC]	1363[B]	1579[B]	5.07[b]	0.96	56[B]
Cecum digesta							
Control	1973	1102	3569	3728	6.81	1.00	312
APSC	1782	972	2948	3275	6.65	0.99	265
GBSC	1679	952	3722	3739	6.47	0.99	348

a, b, P < 0.05; A, B, P < 0.10

β-diversity: PCoA plots APSC, alfalfa-pellet SARA challenge; GBSC, a grain-based SARA challenge. Significance levels unweighted analysis, APSC vs. Control P = 0.01; GBSC vs. Control P < 0.01; GBSC vs. APSC P = 0.15. Significance levels weighted analysis, APSC vs. Control P = 0.22; GBSC vs. Control P < 0.01; GBSC vs. APSC P = 0.06.
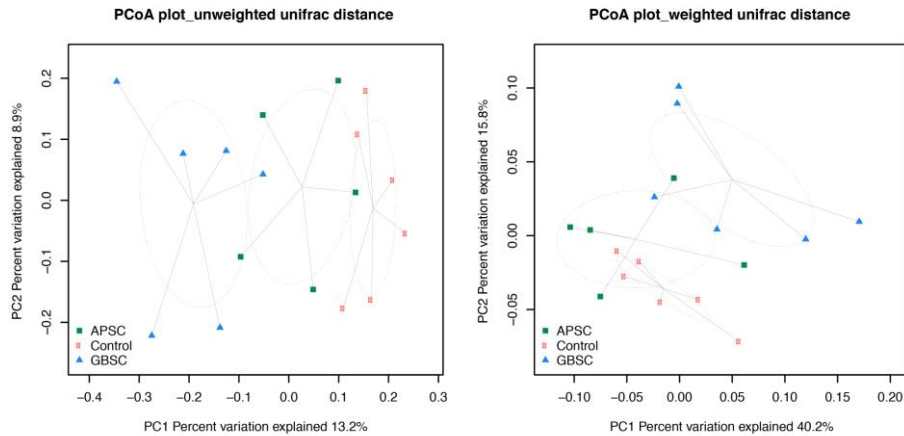

Relative abundance of phyla (above 0.1% of community) in rumen fluid of dairy cows fed a control diet or on cows given an alfalfa pellet SARA challenge (APSC) or a grain-based SARA challenge (GBSC). Bacteria phyla were classified using V1–V3 16S rRNA gene pyrosequencing

Phyla	Percentage of sequences in:			SEM	*P*-value
	Control	APSC	GBSC		
Bacteroidetes	48.9[a]	49.6[a]	41.9[b]	2.3	<0.01
Firmicutes	43.0	41.8	52.2	3.9	0.13
Spirochaetes	3.8	3.3	0.9	2.1	0.19
Tenericutes	1.1[a]	0.9[a]	0.4[b]	0.1	<0.01
Proteobacteria	0.56	0.73	0.30	0.21	0.16
Actinobacteria	0.37	0.26	3.24	1.96	0.58
Fibrobacteres	0.35	0.59	0.32	0.11	0.24
SR1	0.25[aA]	0.14[abB]	0.02[bB]	0.05	0.02
Cyanobacteria	0.18[abA]	0.32[aA]	0.01[bB]	0.08	0.01
TM7	0.10	0.06	0.14	0.06	0.29

a, b, P < 0.05; A, B, P < 0.10

Effects of a grain-based SARA challenge (GBSC) and an alfalfa pellet SARA challenge (APSC) on sixteen bacterial species and a group of Lactobacillus spp. in rumen liquid, cecal digesta, and feces determined by quantitative RT-PCR.
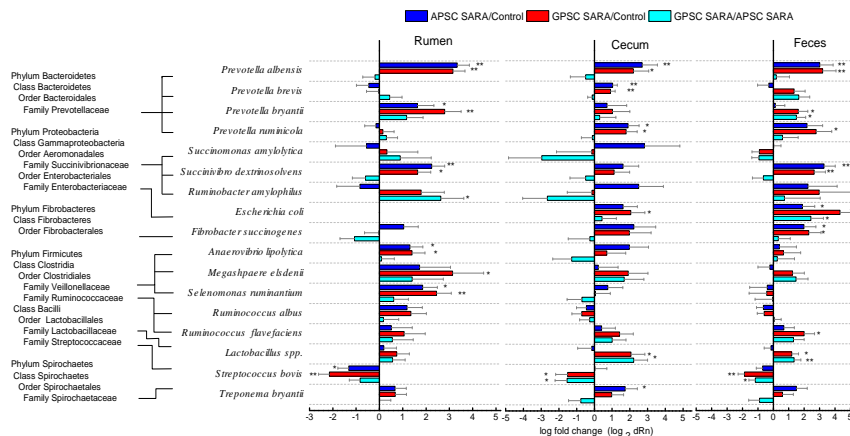
Dysbiosis

- Unbalanced microbial community
- Indicated by high ratio of Proteobacteria to (Firmicutes + Bacteroidetes) ? (Auffret et al., 2017)
- Increased grain/starch feeding alters microbiota in digestive tract, but does this constitute dysbiosis?

SARA in grazing cows

- In grazing cows, high-quality pastures with low NDF content and high moisture contents can cause ruminal pH depression (Westwood et al., 2003).
- Comparing grazing cows with rumen pH < 5.8 and those with rumen pH >5.8 (O'Grady et al. 2008):
  - No difference in milk yield, milk composition, rumen VFA, fecal consistency, and rumen fill
  - Threshold used was pH 5.8 using rumenocentesis, so any SARA?

SARA in grazing cows

- Low ruminal pH (range 5.6 to 6.8) in pasture-fed cows (Kolver and de Veth, 2002)
  - Higher microbial N flow from the rumen, total ruminal VFA (SCFA), milk yield and DMI
  - Lower milk fat percentage, fat:protein ratio, acetate:propionate ratio
  - Any SARA?
  - Low pH = high VFA = More rumen available energy for microbial growth?

Microbial protein

- Microbial protein supplies 60 to 85% of amino acids reaching the small intestine (SI) (Storm et al., 1983)
  - Efficient?

- Optimal protein supply to the SI depends on adequate degradable protein providing **N (ammonia, amino acids and peptides)** as well as **ENERGY** for microbial growth.

- Enhancing the efficiency of microbial protein (microbial mass) production would improve utilization of dietary protein/N and reduce excretion of N.

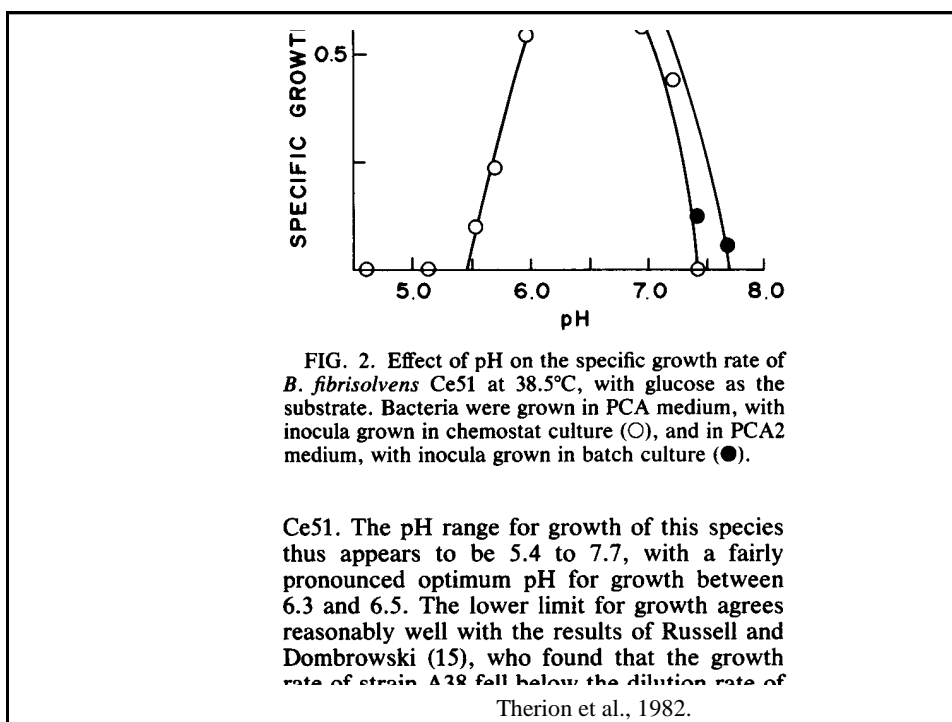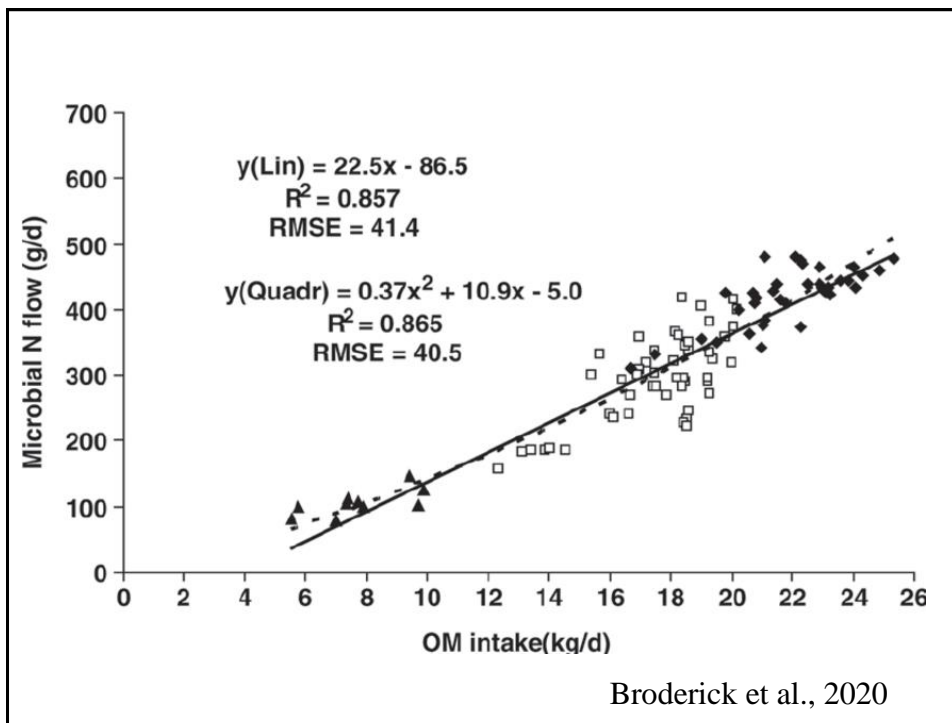## Efficiency of microbial protein production

Organism	Efficiency	
	g microbial DM (mol ATP)$^{-1}$[a]	% of theoretical maximum[b]
Mixed rumen microbes, *in vivo*	11–21	34–66
Mixed rumen bacteria, *in vitro*	7.5–16.7	23–52
Pure cultures, *in vitro*	10–25	31–78

[a] Summarized from Russell and Wallace (1997).
[b] 31.9 g (g microbial DM mol ATP)$^{-1}$; value from Stouthamer (1973) for growth with glucose, amino acids, and nucleic acid bases.

Hackmann and Firkins, 2015

Maximum at 12% DM RDP (NRC, 2021)

y(Lin) = 22.5x - 86.5
R^2 = 0.857
RMSE = 41.4

y(Quadr) = $0.37x^2$ + 10.9x - 5.0
R^2 = 0.865
RMSE = 40.5

Broderick et al., 2020

FIG. 2. Effect of pH on the specific growth rate of
B. fibrisolvens Ce51 at 38.5°C, with glucose as the
substrate. Bacteria were grown in PCA medium, with
inocula grown in chemostat culture (○), and in PCA2
medium, with inocula grown in batch culture (●).

Ce51. The pH range for growth of this species
thus appears to be 5.4 to 7.7, with a fairly
pronounced optimum pH for growth between
6.3 and 6.5. The lower limit for growth agrees
reasonably well with the results of Russell and
Dombrowski (15), who found that the growth
rate of strain A38 fell below the dilution rate of

Therion et al., 1982.

Microbial protein

- Production of microbial protein is inefficient because microbes direct a proportion of available energy toward maintenance functions, synthesis of reserve carbohydrate, and energy spilling (Hackmann and Firkins, 2015).
- A reduction of the pH from 6.7 to 5.7 reduced *in vitro* protein microbial synthesis by 73% (Strobel and Russel, 1986).
- A reduction of the rumen pH depresses fibrolytic and proteolytic bacteria, but fermentation of starches and sugars remain very high.

Solutions?

- Ensure that the composition of the ingested diet (especially coarse fibre and grain) resembles that of the formulated diet.
  - Prevent sorting and mixing errors (Miller-Cushon and DeVries, 2017)
- Allow multiple smaller meals rather than fewer large meals
  - Prevent empty bunks (de Vries, 2019).
  - Sufficient time/space at feed bunk (de Vries, 2019).
  - Avoid competition at the feed bunk (de Vries, 2019).
- Feed according to individual animal requirements
  - Precision feeding
  - How to assess individual requirements? (milk urea nitrogen MUN)?

Solutions?

- Feed the microbes!
- Yeast and yeast culture products, especially those derived from *Saccharomyces cerevisiae*, can stabilize the conditions in the foregut and hindgut of cattle during high grain feeding (Al Ibrahim et al., 2012; Chiquette et al., 2015; Li et al., 2016)
- Direct-fed microbials, e.g. *Enterococcus faecium* and *Lactococcus lactis,* and polyphenols, have also shown promise to attenuate SARA (De Nardi et al., 2014; Chiquette et al., 2015)
- Yeasts
  - Live
  - Dead
  - Yeast culture fermentation products (Dead yeast, remaining medium, metabolites)

Effects of active dry *Saccharomyces cerevisiae* supplementation on microbial community during subacute ruminal acidosis (SARA) determined by qRT-PCR
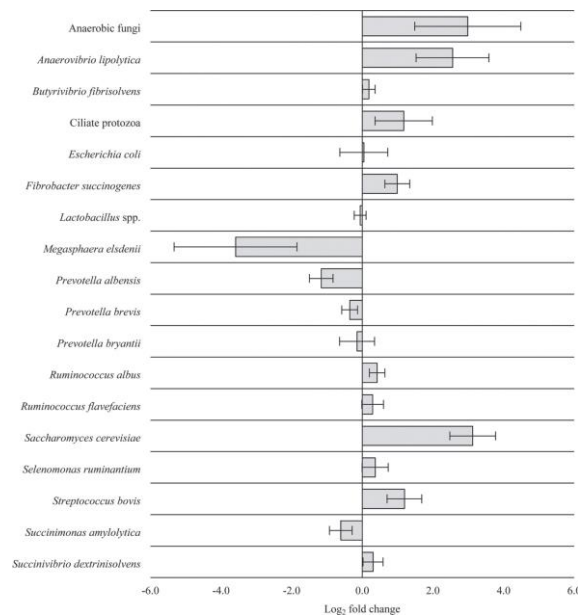
Al-Zahal et al., 2014

Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) and grain-based subacute ruminal acidosis (SARA) on biodiversity indices of bacterial communities in rumen fluid

| Item	No SCFP		SCFP		SEM	Significance (***P***-value)		
	Control	SARA	Control	SARA		SARA	SCFP	SARA *SCFP
Number of reads	2557	3927	3126	4697	833	0.10	0.44	0.91
Observed Species	197	128	196	174	14	0.01	0.14	0.12
Chao1	741	337	643	548	59	<.001	0.45	0.01
ACE	839	359	779	612	75	<0.01	0.30	0.02
Shannon	5.88	4.91	5.88	5.53	0.19	<0.01	0.17	0.06
Simpson	1.97	1.93	1.98	1.97	0.01	<0.01	0.20	0.08
InvSimpson	63	22	65	50	11	<0.01	0.22	0.19

Source: Plaizier et al., 2016

Effects of a *Saccharomyces cerevisiae* fermentation product (SCFP) and grain-based subacute ruminal acidosis (SARA) on the relative abundances of major phyla

Phylum	No SCFP		SCFP		SEM	Significance (***P***-value)		
	Control	SARA	Control	SARA		SARA	SCFP	SARA *SCFP
	------------- above 0.1% population -----------							
Bacteroidetes	46.84	20.78	40.57	32.49	3.72	<0.01	0.44	0.02
Firmicutes	35.08	53.86	43.44	50.25	5.06	0.04	0.69	0.30
Proteobacteria	1.12	13.88	0.78	5.50	2.74	0.02	0.39	0.46
Spirochaetes	1.41	0.11	1.79	0.96	0.22	<0.01	0.06	0.68
Tenericutes	1.85	0.45	1.84	1.06	0.24	<0.01	0.24	0.22
Cyanobacteria	1.05	0.41	0.60	0.44	0.15	0.02	0.23	0.22
SR1	0.75	0.43	0.53	0.22	0.20	0.14	0.32	0.18
TM7	0.41	0.28	0.31	0.23	0.08	0.22	0.36	0.99
Verrucomicrobia	0.23	0.02	0.36	0.04	0.05	<0.01	0.17	0.74

Source: Plaizier et al., 2016

Experimental design
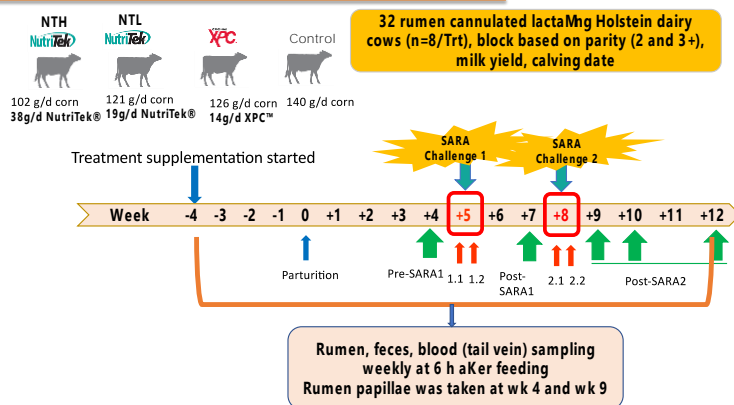
Table 4. Effect of *Saccharomyces cerevisiae* fermentation products (SCFP) treatments (control, SCFPa, SCFPb-1X, and SCFPb-2X) by stages of subacute ruminal acidosis (SARA; pre-SARA1, SARA1, post-SARA1, SARA2, and post-SARA2) on rumen time < pH 5.6.

Stage	Treatment				SEM	Effects, P
	Control	XPC	NTL	NTH		
Pre-SARA1	7.1	3.2	2.9	6.3	3.6	0.60
SARA1	228.4a	183.1ab	241.0a	104.6b	64.1	0.01
Post-SARA1	26.58	6.28	24.08	4.26	10.1	0.10
SARA2	121.6b	284.4a	252.8a	53.9b	45.4	<0.001
Post-SARA2	14.6	27.6	3.1	21.3	9.7	0.32

Note: Means with different lowercase letters (a and b) within SARA stage differ ($P < 0.05$). SEM, standard error of mean.

Effect of second challenge on rumen pH not more severe than first

Repeated SARA challenges

- Cows exposed to 3 1-day grain–based SARA challenges, each separated by 14 d (Dohme et al., 2008)
  - with each successive challenge, the rumen pH depression was more severe.
- Two 28 d grain-based SARA challenges separated by 6 d baseline and 6 d of grain adaptation (Qumar et al., 2008)
  - More severe rumen pH depression during second challenge

Both SARA challenges and SCFP affected rumen fluid microbial composition– Beta-diversity
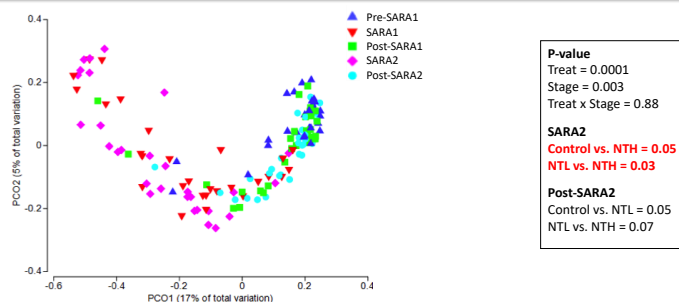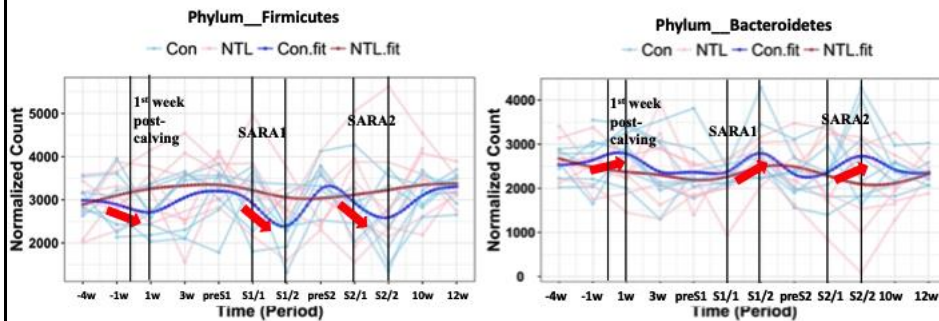

Figure1. Principal coordinates analysis (PCoA) of Bray-Curtis distances of **rumen liquid** microbial communities.
(a) Bray-Curtis distances were used to compare the composition of microbiota among treatments (Control, XPC, NTL and NTH).
(b) Bray-Curtis distances were used to compare the composition of microbiota among treatments different stages of SARA induction (Pre-SARA1, SARA1, Post-SARA1, SARA2, Post-SARA2).
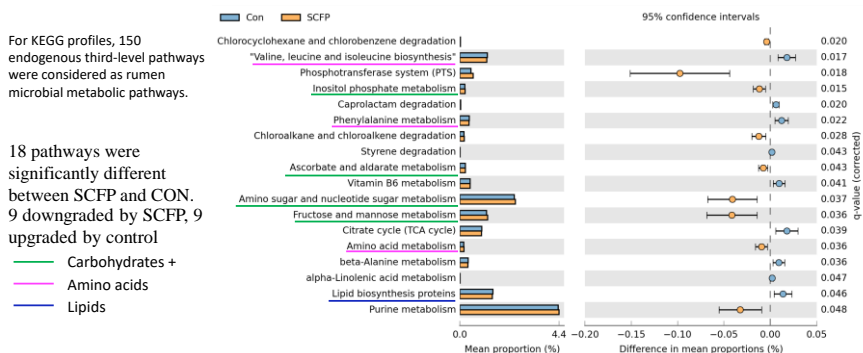
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SCFP **stabilizes** rumen fluid microbiome as lactation progress

Phylum__Firmicutes
— Con — NTL — Con.fit — NTL.fit

Phylum__Bacteroidetes
— Con — NTL — Con.fit — NTL.fit

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Changes in predicted functionality determined with CowPi

Differences of predicted microbial functions between SCFP and Con

For KEGG profiles, 150 endogenous third-level pathways were considered as rumen microbial metabolic pathways.

18 pathways were significantly different between SCFP and CON. 9 downgraded by SCFP, 9 upgraded by control

— Carbohydrates +
— Amino acids
— Lipids

Con   SCFP   95% confidence intervals

Pathway	q-value (corrected)
Chlorocyclohexane and chlorobenzene degradation	0.020
"Valine, leucine and isoleucine biosynthesis"	0.017
Phosphotransferase system (PTS)	0.018
Inositol phosphate metabolism	0.015
Caprolactam degradation	0.020
Phenylalanine metabolism	0.022
Chloroalkane and chloroalkene degradation	0.028
Styrene degradation	0.043
Ascorbate and aldarate metabolism	0.043
Vitamin B6 metabolism	0.041
Amino sugar and nucleotide sugar metabolism	0.037
Fructose and mannose metabolism	0.036
Citrate cycle (TCA cycle)	0.039
Amino acid metabolism	0.036
beta-Alanine metabolism	0.036
alpha-Linolenic acid metabolism	0.047
Lipid biosynthesis proteins	0.046
Purine metabolism	0.048

0.0   Mean proportion (%)   4.4   −0.20 −0.15 −0.10 −0.05 0.00 0.05
Difference in mean proportions (%)

31

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Both SARA challenges and SCFP affected rumen solid microbial composition– Beta-diversity

Legend:
- Pre-SARA1
- SARA1
- Post-SARA1
- SARA2
- Post-SARA2

P-value
Treat = 0.0004
Stage = 0.0001
Treat x Stage = 0.95

SARA2
NTL vs. NTH = 0.04
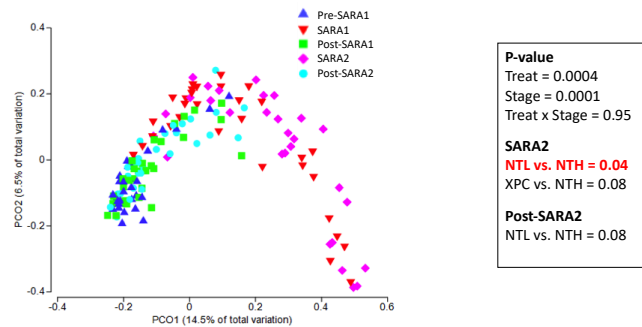XPC vs. NTH = 0.08

Post-SARA2
NTL vs. NTH = 0.08

Figure2. Principal coordinates analysis (PCoA) of Bray-Curtis distances of **rumen solids** microbial communities.
(a) Bray-Curtis distances were used to compare the composition of microbiota among treatments (Control, XPC, NTL and NTH).
(b) Bray-Curtis distances were used to compare the composition of microbiota among treatments different stages of SARA induction (Pre-SARA1, SARA1, Post-SARA1, SARA2, Post-SARA2).

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No treatment effect on rumen solid microbiome (abundant members)as lactation progress
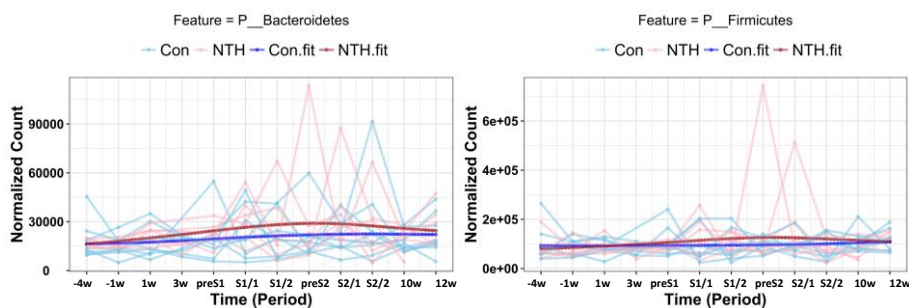
Similar patterns were found in NTL vs Con and XPC vs Con.

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## Changes in predicted functionality determined with CowPi

55 pathways were significantly different between SCFP and CON.

27 pathways were enriched in SCFP, including 6 in carbohydrate metabolism, 1 in Lipid and 2 in Amino Acid metabolism.

8 pathways in Amino Acid Metabolism, 1 in Lipid metabolism and 2 in Carbohydrate metabolism were enriched in Con.

— Carbohydrates +
— Amino acids
— Lipids
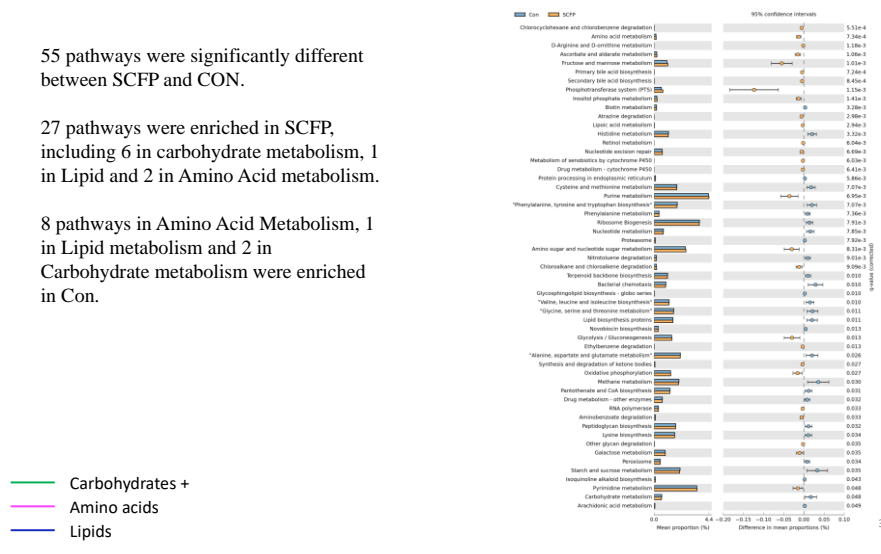
Conclusions

- Gut health is multifactorial, and involves chemical and physical composition of digesta, functionality of microbiota, and functionality of mucosa
- How representative is grain-induced SARA for for SARA occurring on dairy farms?

Conclusions

- At the bacterial phylum level the rumen microbiome appears robust to grain-induced SARA.
- At the bacterial species level, grain-induced SARA affects the abundances of many common rumen bacteria in ways reflecting changes in substrate availabilities and competition.

Conclusions

- Meta-genomics and meta-transcriptomics are needed to comprehensively study effects of dietary interventions on microbiota in the rumen.
- Feed supplements that attenuate adverse impact of high grain feeding, e.g. yeast and yeast culture products, are available.

Thank you for your attention

Questions/Vragen?

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