

For Better or Worse: How Rations Affect Ruminal Microbial Populations



Paul Weimer Lab:
David Stevenson
Chris Odt
David Welkie
Alberto Palmonari

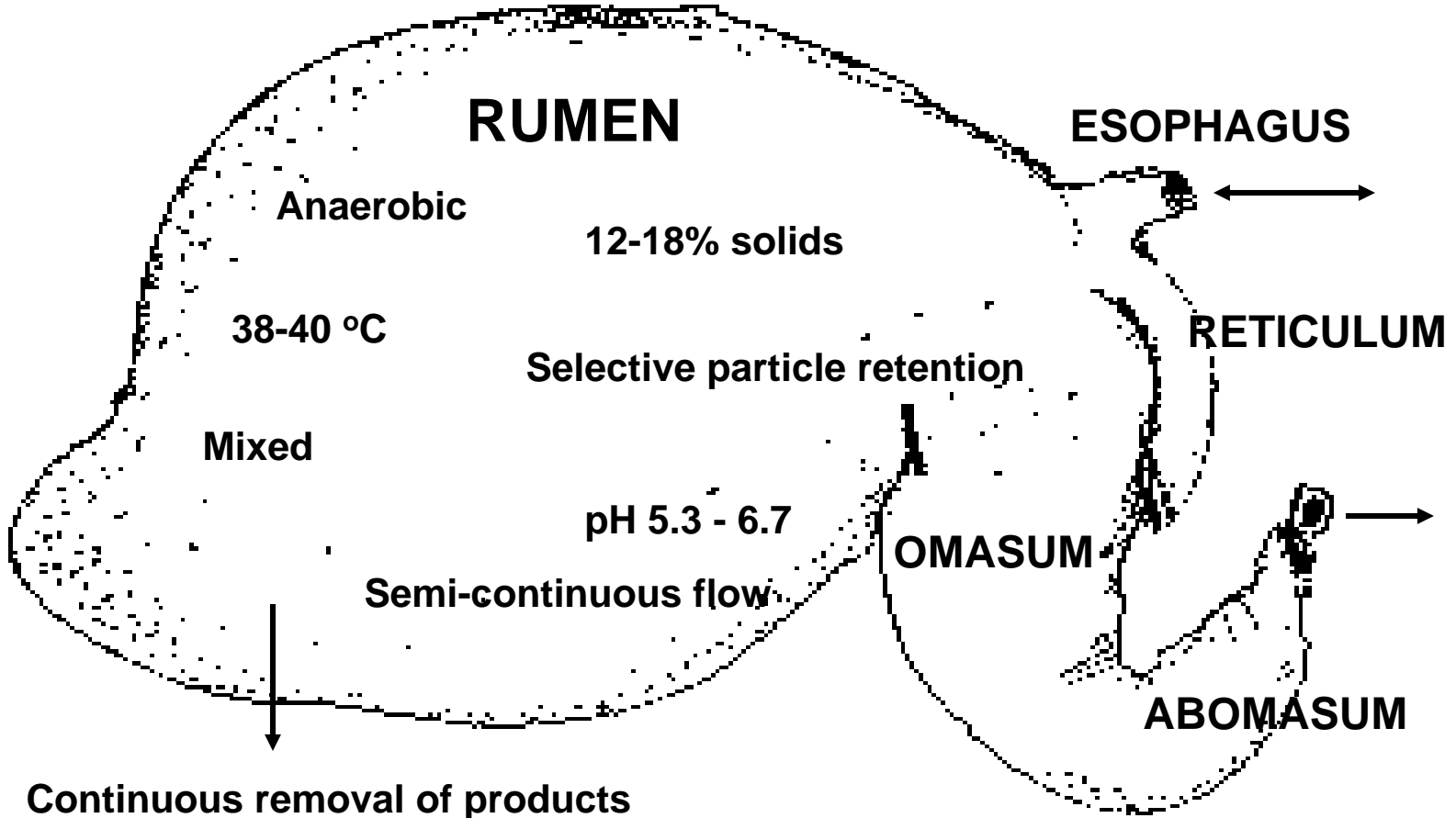
World Dairy Expo Oct. 2, 2008



Outline



- **The rumen and its microbes**
- **Cultured vs. non-cultured populations**
- **Molecular analysis of bacterial populations**
- **Two case studies**
 - **Shifts during the feeding cycle**
 - **Shifts associated with milk fat depression**



RUMEN

ESOPHAGUS

Anaerobic

12-18% solids

38-40 °C

Selective particle retention

RETICULUM

Mixed

pH 5.3 - 6.7

OMASUM

Semi-continuous flow

ABOMASUM

Continuous removal of products

“VFAs” (Acetic, Propionic, Butyric acids)





Types of Rumen Microorganisms

BACTERIA

Ferment fiber, starches and sugars in feeds to VFA, H₂ and CO₂

Produce most of microbial cell protein, but also ferment feed proteins to VFA + NH₃

PROTISTS

Consume and ferment bacteria to VFA + NH₃

Sequester and ferment starch

Recycle N

ARCHAEA

Convert H₂ and CO₂ to methane

FUNGI

Assist in fiber digestion

Rumen Bacteria

> 1 billion bacterial cells per drop of rumen fluid!

Can be classified into different types or “species”

Species are grouped based on their shared evolutionary history and on details of their cell structure and metabolism

Division



Class



Order



Family



Genus



Species

How many different species of rumen bacteria?

Culture-dependent approach

Use laboratory culture methods to isolate different species in pure culture, and determine characteristics of each species.

Culture-independent approach

Identify species in rumen samples using each species' "molecular signature", without having to isolate the species in pure culture.

Only ~ two dozen species of bacteria can be isolated from most rumen samples.

Studies in non-rumen environments suggest that only a small fraction (1 to 10 %) of the species are culturable.

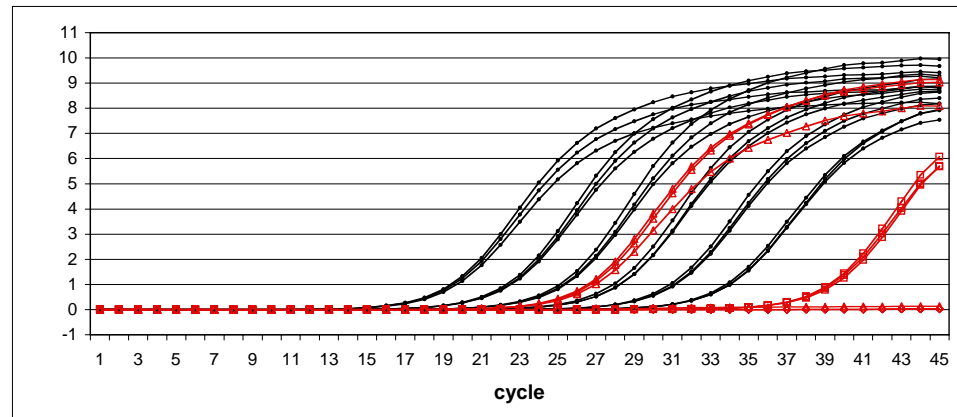
Counting Bacterial Groups in the Rumen

How abundant or important are individual bacterial species that have provided the basis for our understanding of rumen microbiology?

Extract and purify DNA

Amplify by real-time PCR using taxon-specific primers directed to 16S rRNA genes

Quantify against standards to determine a “Relative Population Size” (RPS)



“Classical” Rumen Bacterial Species are Not Abundant

Data from rumen samples (combined liquid and solid phases)
collected 6 h post feeding

% of Bacterial 16S rRNA gene copy number

Target taxon	Cow 4884		Cow 4991	
	Day 30	Day 31	Day 30	Day 31
<i>Butyrivibrio fibrisolvens</i>	0.0216	0.0273	0.0220	0.0243
<i>Eubacterium ruminantium</i>	0.1707	0.1581	0.1634	0.2130
<i>Fibrobacter succinogenes</i>	0.8384	0.8889	0.6152	0.9954
<i>Megasphaera elsdenii</i>	0.0011	0.0001	0.0003	0.0004
<i>Prevotella brevis</i>	0.1616	0.0988	0.1524	0.1282
<i>Prevotella bryantii</i>	1.226	0.7296	1.942	1.830
<i>Prevotella ruminicola</i>	1.600	1.5822	1.756	2.032
<i>Ruminobacter amylophilus</i>	0.1697	0.1406	0.3920	0.189
<i>Ruminococcus albus</i>	0.0030	0.0013	0.0044	0.0076
<i>Ruminococcus flavefaciens</i>	0.7573	0.3357	0.5580	0.7993
<i>Selenomonas ruminantium</i>	0.7061	0.3412	0.4681	0.6880
<i>Streptococcus bovis</i>	0.0077	0.0021	0.0025	0.0023
<i>Succinivibrio dextrinosolvens</i>	0.7148	0.6560	1.071	0.7988
Sum of individual species	6.186	4.920	6.900	7.213
Genus <i>Prevotella</i>	49.60	42.44	58.12	59.93

Most studied rumen
bacteria to date

Automated Ribosomal Intergenic Spacer Analysis

- A culture-independent, “community fingerprinting” method that captures full spectrum of bacterial diversity within sample.



- contains tRNA genes and noncoding sequences
- size varies with bacterial species (~130 to ~1500 nt)

Each discrete segment length is assumed to correspond to an individual bacterial “species” (Operational Taxonomic Unit, OTU)

ARISA of Bacterial Community Composition



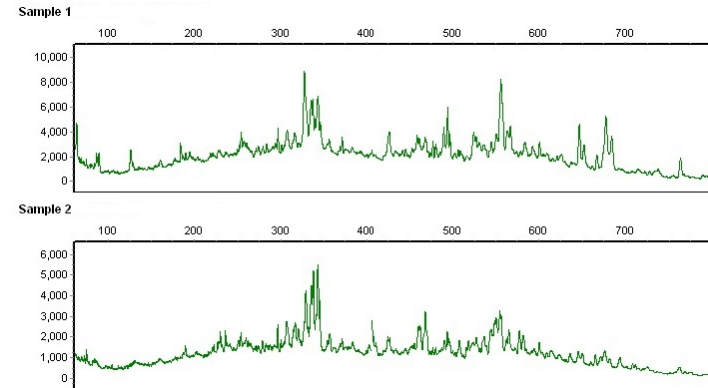
Rumen sampling



Separate solid and liquid fractions

Extract and purify DNA

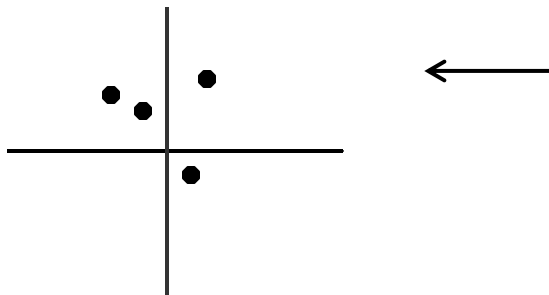
PCR-amplify ITS region between
16S and 23S rRNA genes



Separate by capillary
electrophoresis



ITS length	samp 1	samp 2	samp 3	samp 4
461	0	0.6960	1.1559	1.3742
462	0	0	0	2.2501
468	0	0	2.0226	2.336
472	0	0	0	0
474	0.6882	0.5221	1.0031	1.2493
477	0.9635	1.0141	1.4755	1.8457
480	0.6669	1.4532	2.1095	2.6771
482	0	0	0	0
484	0.572	0.5045	0.9139	1.0761

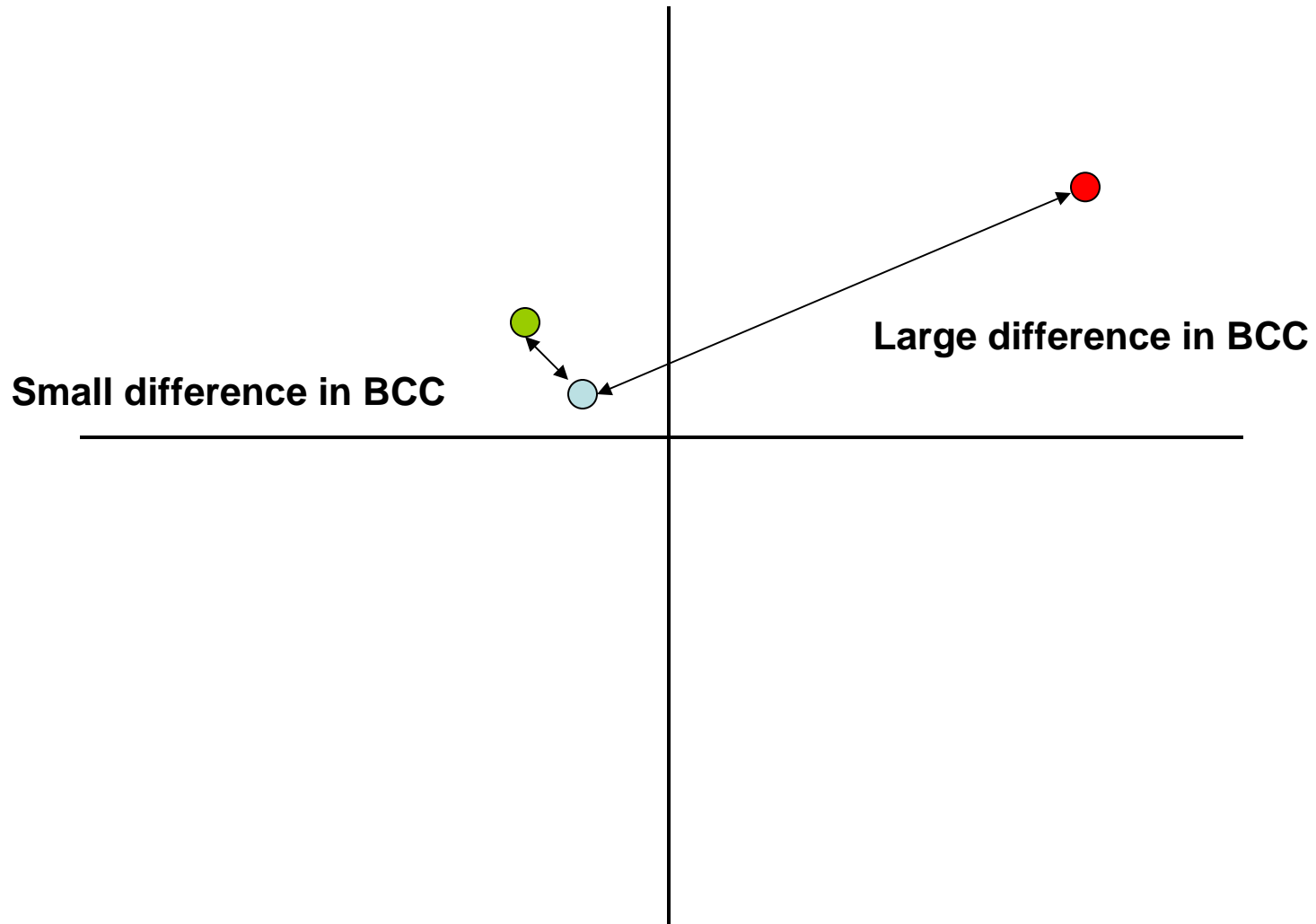


Multivariate Statistical
Analysis (PCA, CA, etc.)

Construct
data matrix

~ 100 to 200 rows (OTUs)
2 columns for each rumen
sample (solid & liquid)

Correspondence Analysis of Bacterial Community Composition



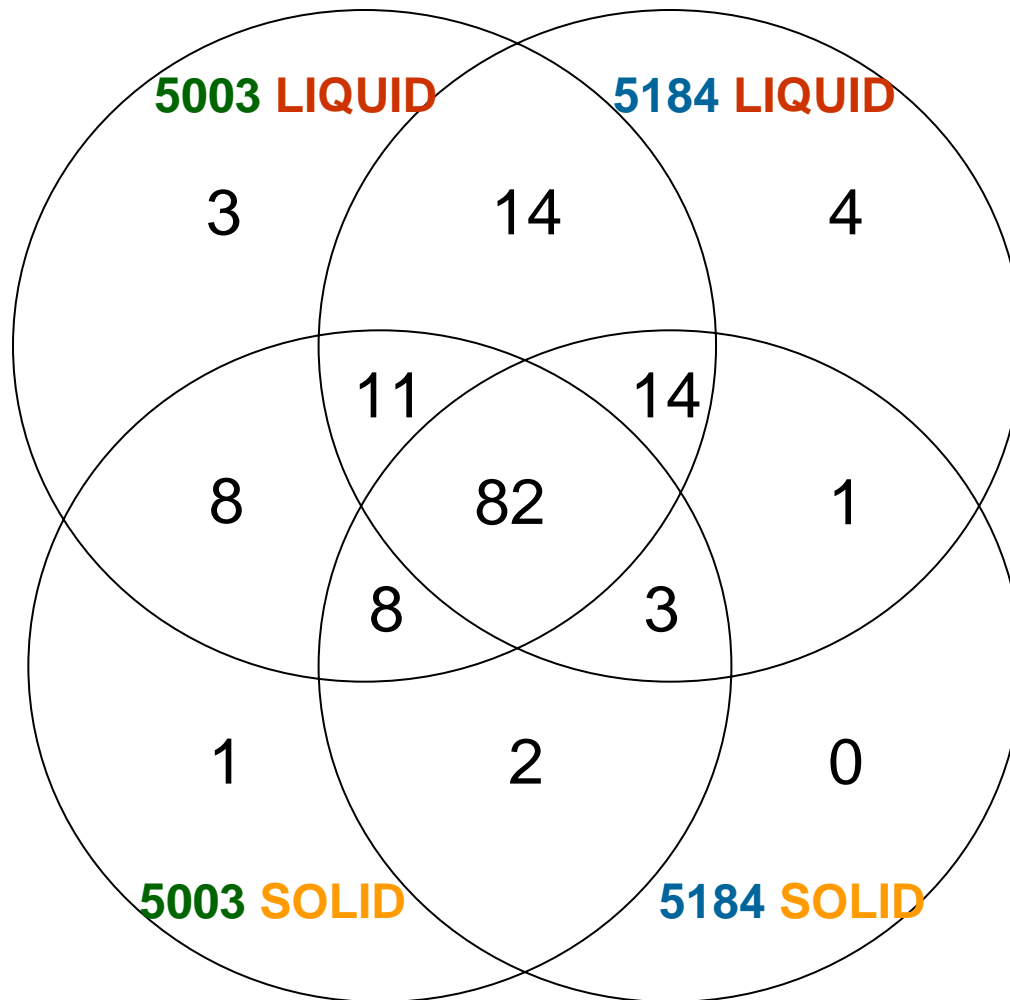
Do different cows harbor different rumen bacterial populations?

Is the analysis affected by sampling time?

Experiment:

- **2 cows fed TMR (alfalfa haylage + corn silage + SBM) at 12 h intervals.**
- **Rumen samples collected at 2, 4, 6, 9 and 12 hrs after feeding over 4 feeding cycles.**
- **Samples analyzed by ARISA to determine bacterial community composition (BCC).**

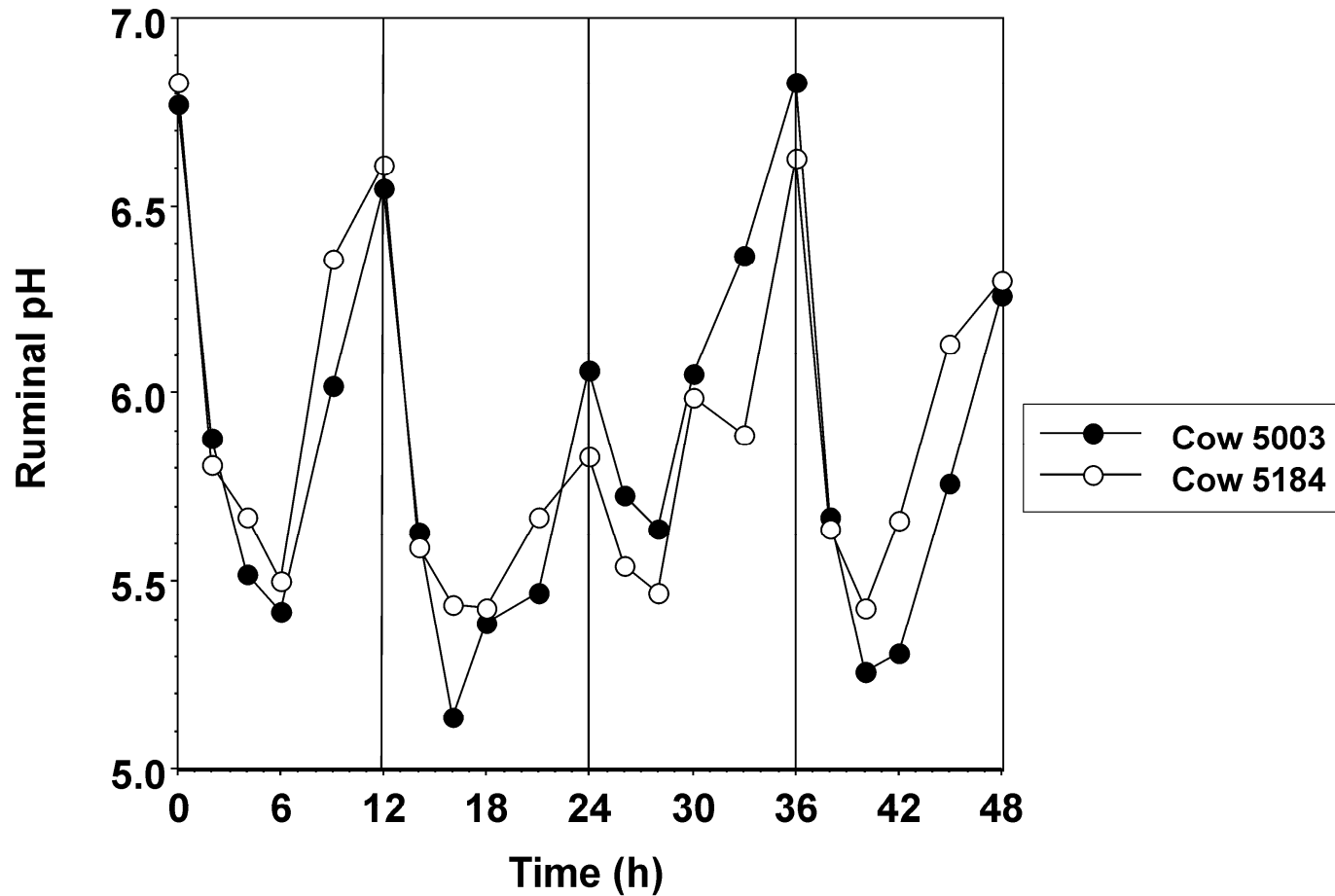
Numbers of OTUs (Operational Taxonomic Unit) detected by cow and phase



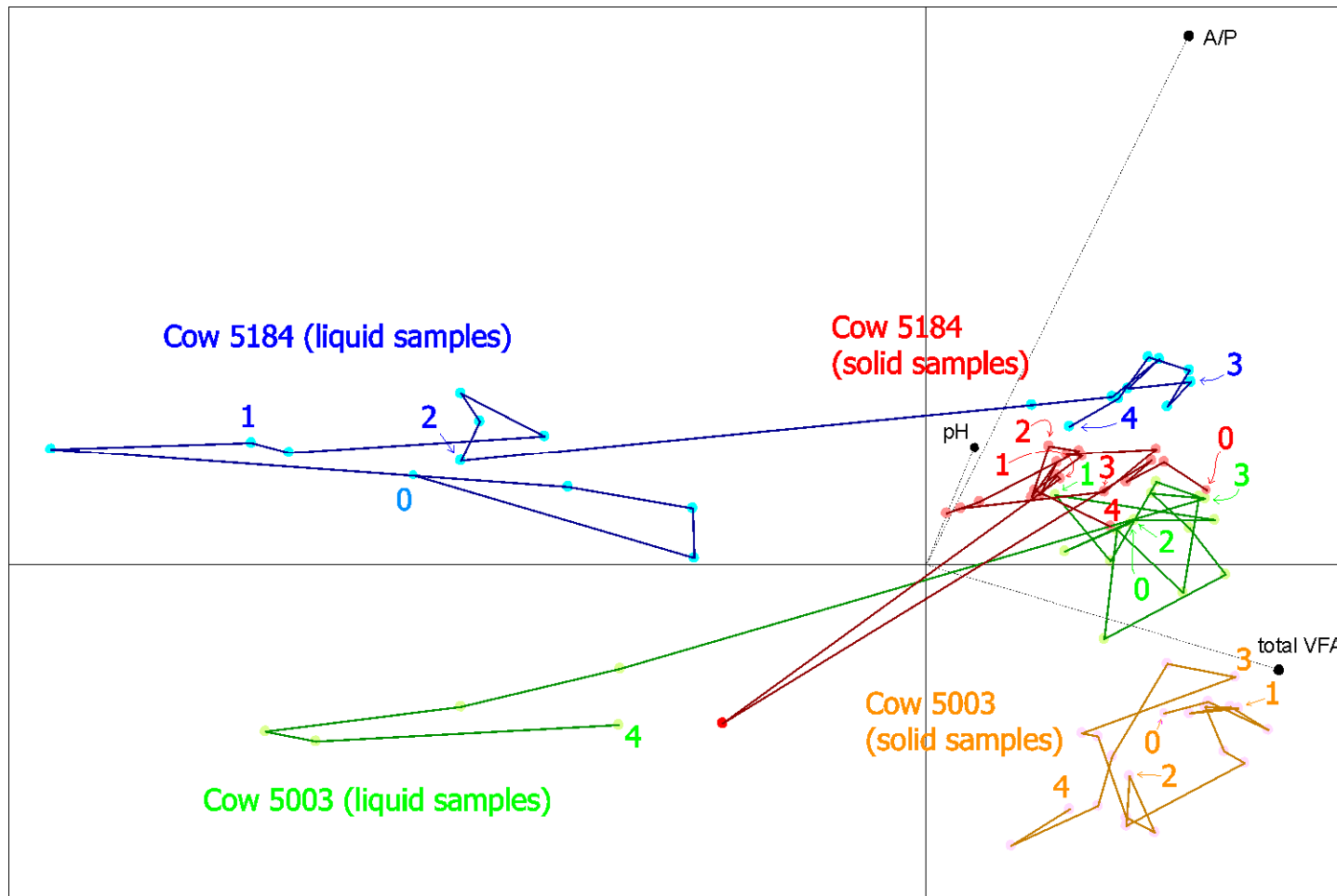
n=155

4 OTUs were present in opposite phases in the two cows

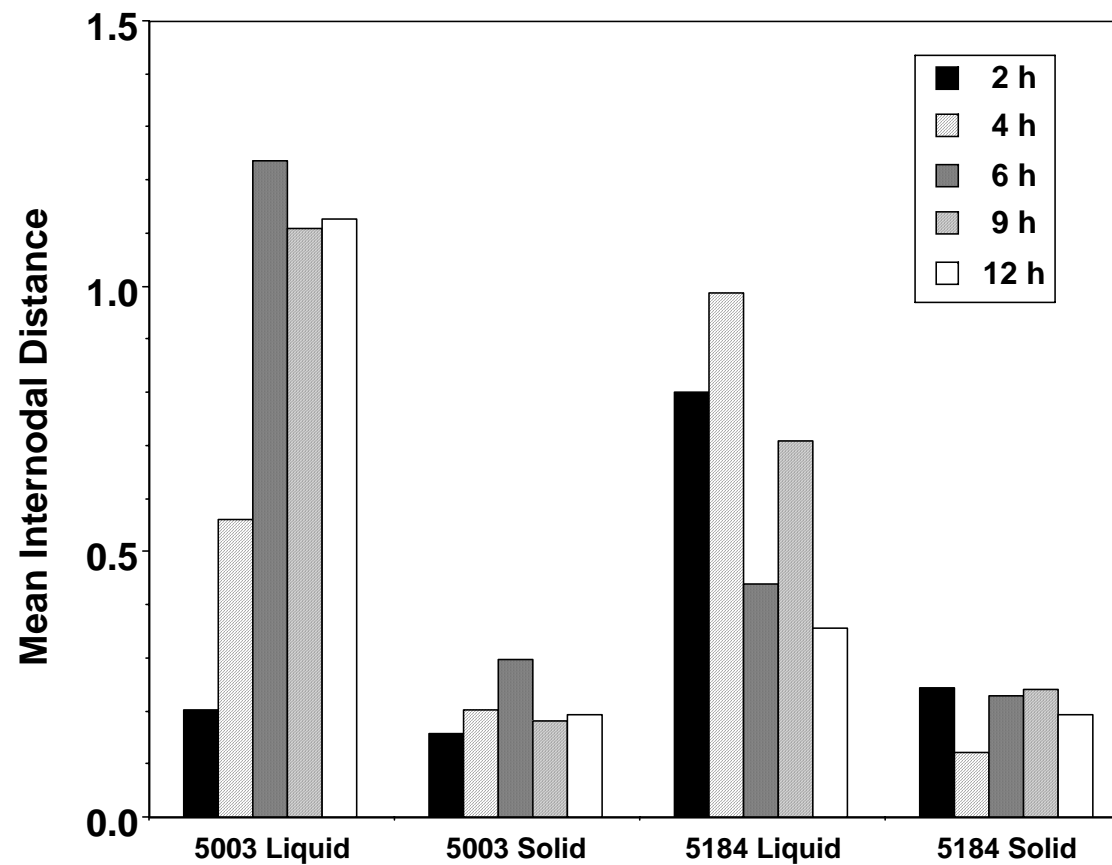
pH Changes through 4 Feeding Cycles



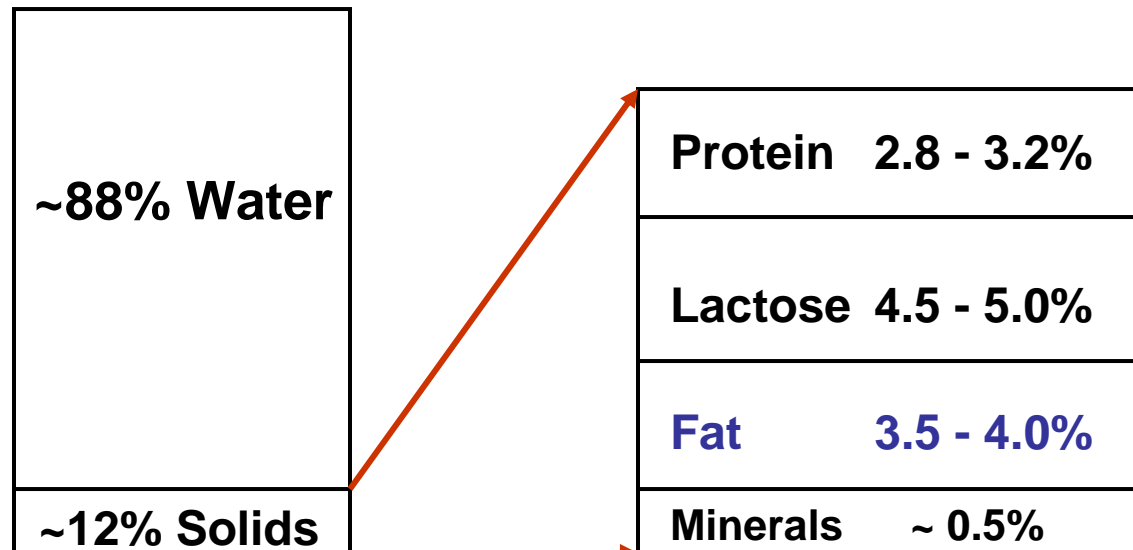
Bacterial Community Composition changes through 4 feeding cycles



Population Changes across Feeding Cycles



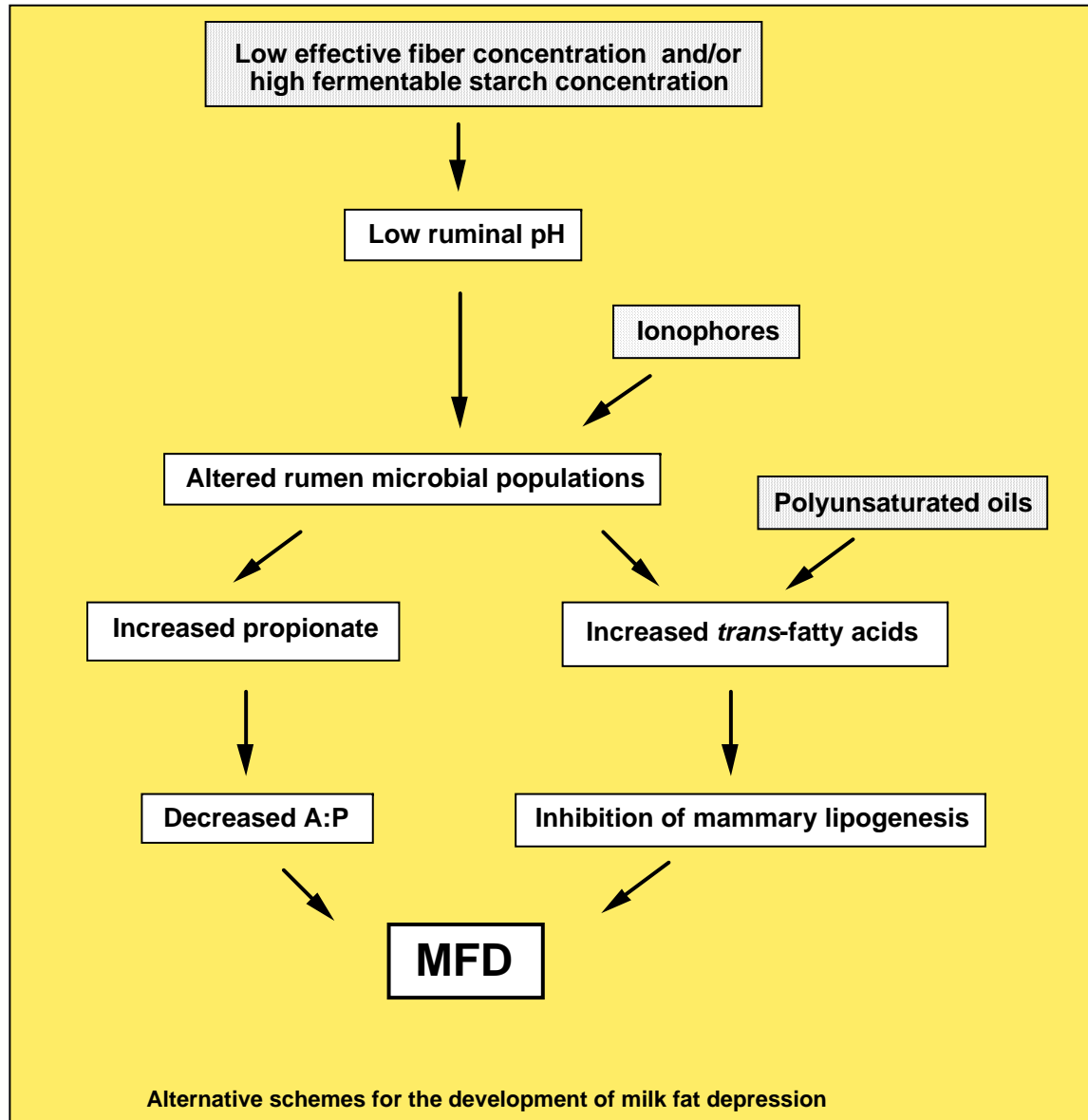
Effect of Rumen Microbial Populations on Milk Composition



Milk value largely determined by its fat content.



Reduced fat content, or “milk fat depression” (MFD), is a major economic loss to dairy producers.

Proposed origins of MFD





No definitive relationship has been established between milk fat depression (MFD) and microbial populations in the rumen

- **Can we relate MFD to changes in bacterial community composition?**
 - **Can we identify individual bacterial species whose populations change substantially under conditions of MFD?**
- 
- 

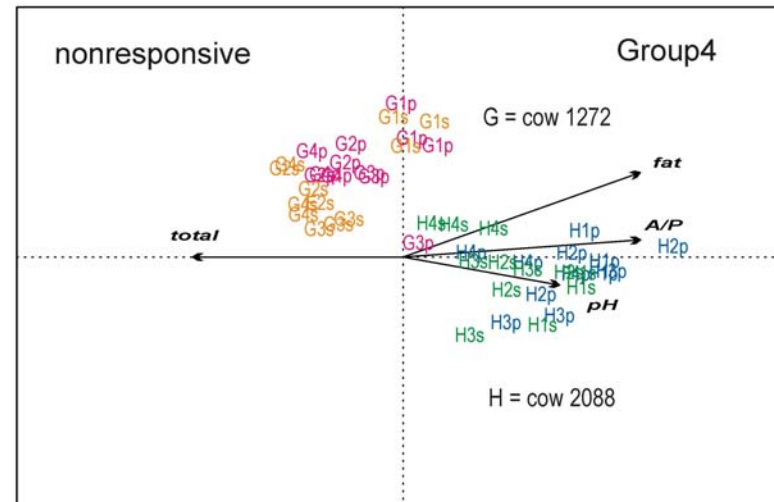
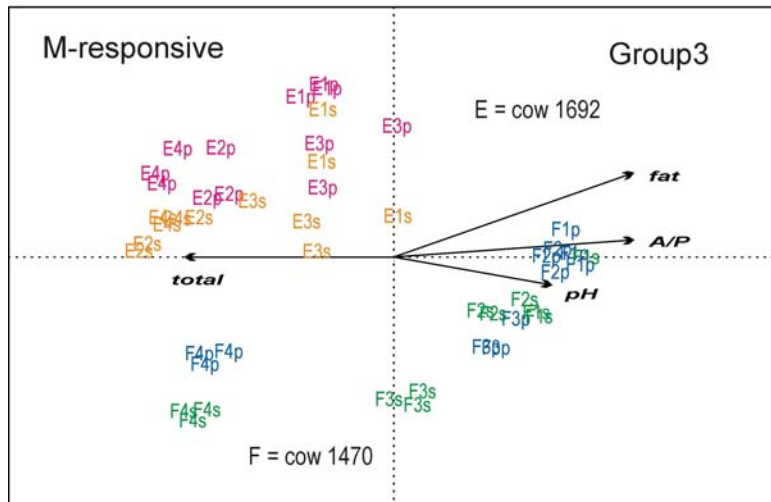
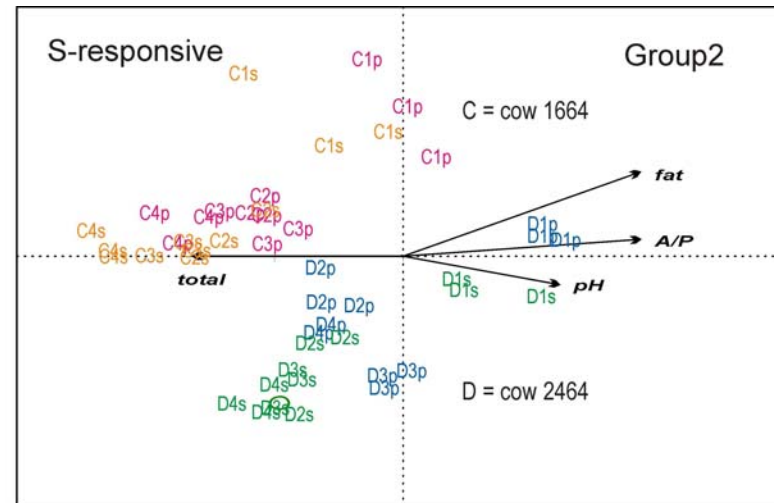
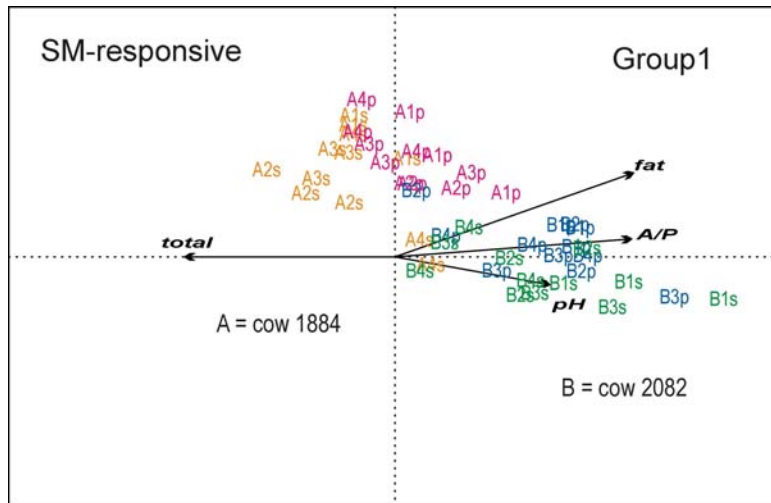
Milk fat Depression Study

(funded by Elanco Animal Health, Inc.)

- 18 lactating Holstein cows
- 4 sequential dietary treatments (28 d each) based on alfalfa haylage/corn silage/soybean meal supplemented with:
 - Period 1: dry corn grain SFS
 - Period 2: high-moisture corn RFS
 - Period 3: high-moisture corn + monensin RFS/Mon
 - Period 4: high-moisture corn RFS/Post
- Archived rumen samples collected 6 h after feeding on last 3 days of each period
- Identified 4 pairs of cows that differed in milk fat response
- Analyzed Bacterial Community Composition in those 8 cows

Cows grouped by milk fat response

Dietary Treatment	Cow	Group 1 (SM-responsive)		Group 2 (S-responsive)		Group 3 (M-responsive)		Group 4 (Non-responsive)	
		1884	2082	1664	2464	1272	2088	1692	2470
% Fat:									
SFS		4.25	4.27	3.43	3.68	3.61	3.49	3.53	3.76
RFS		3.92	3.80	2.98	2.98	3.65	3.68	3.38	3.47
RFS/Mon		3.51	3.80	2.43	2.80	2.59	2.58	3.59	3.57
RFS/Post		4.01	4.12	2.70	2.63	2.67	2.60	3.51	3.35
Fat yield (kg/d):									
SFS		1.89	1.24	1.72	1.36	1.80	1.47	1.69	1.42
RFS		1.12	0.99	1.04	1.09	1.51	1.01	1.43	1.25
RFS/Mon		1.23	0.86	1.11	1.01	1.03	0.83	1.53	1.32
RFS/Post		1.45	1.03	0.98	0.98	1.49	1.10	1.43	1.52



7 OTUs increase in cows that display MFD

Operational taxonomic units (OTU) corresponding to individual bacterial species whose mean relative ARISA peak areas in SM-, S-, or M-responsive cows increased relative to those in milk fat non-responsive cows.

<u>Amplicon Length (bp)</u>	<u>% of total pk area in non-responsive cows</u>	<u>Ratio of relative peak area between cow groups</u>		
		<u>SM/N</u>	<u>S/N</u>	<u>M/N</u>
246	0.047	19.08	36.90	45.82
256	0.37	1.57	2.02	1.54
275	0.069	4.14	3.85	3.01
349	0.013	2.16	22.90	12.49
403	0.080	2.33	4.69	1.53
617	0.009	1.90	6.98	9.14
708	0.053	1.60	4.28	5.72

Response of OTU246 to diet varied by cow

Effect of dietary treatment on relative population size of OTU246 in ruminal contents of cows from different milk fat response groups.

<u>Cow group</u>	<u>Percentage of total amplicon peak area ^a</u>			
	<u>SFS</u>	<u>RFS</u>	<u>RFS/Mon</u>	<u>RFS/Post</u>
SM-responsive	0 ^c	2.77 ^b	0.05 ^c	0.79 ^b
S-responsive	0.07 ^c	1.72 ^b	2.51 ^b	2.70 ^b
M-responsive	0 ^c	2.03 ^b	2.10 ^b	4.56 ^b
Non-responsive	0	0.10	0.04	0.05

^a Expressed as percentage of total peak area present as the OTU246 amplicon in the ARISA electropherogram. Data are averaged across 12 samples (2 cows x 2 phases x 3 sampling days) within each dietary treatment.

^{b,c} Mean values within rows having different superscripts differ ($P < 0.05$).

Conclusions



Cows are individuals that are “married” to their rumen microflora

- Measurable differences in **Bacterial Community Composition** are observed in cows fed same diet.
- **BCC** profiles change within and across feeding cycles but do not overlap with those of other cows.

Conclusions



- **Cows differ in milk fat response, and in conditions inducing milk fat depression.**
- **Milk fat depression is associated with changes in Bacterial Community Composition.**
- **Several members of the bacterial community (e.g., OTU246) display substantial changes in relative population size under conditions of milk fat depression and are promising candidates for investigating their potential to cause MFD.**