

# RuminOmics Regional Workshop

Improving efficiency of production  
and reducing environmental  
impact



## Tools for rapid analysis of Phenotypes and the Microbiome

Kevin J. Shingfield

kes14@aber.ac.uk

**RuminOmics: Connecting the animal genome, the intestinal microbiome and nutrition to enhance the efficiency of ruminant digestion and to mitigate the environmental impacts of ruminant livestock production**

**Project legacy: Identification of proxies and tools for large scale phenotyping for genomic selection, optimised nutrition and better management on-farm**

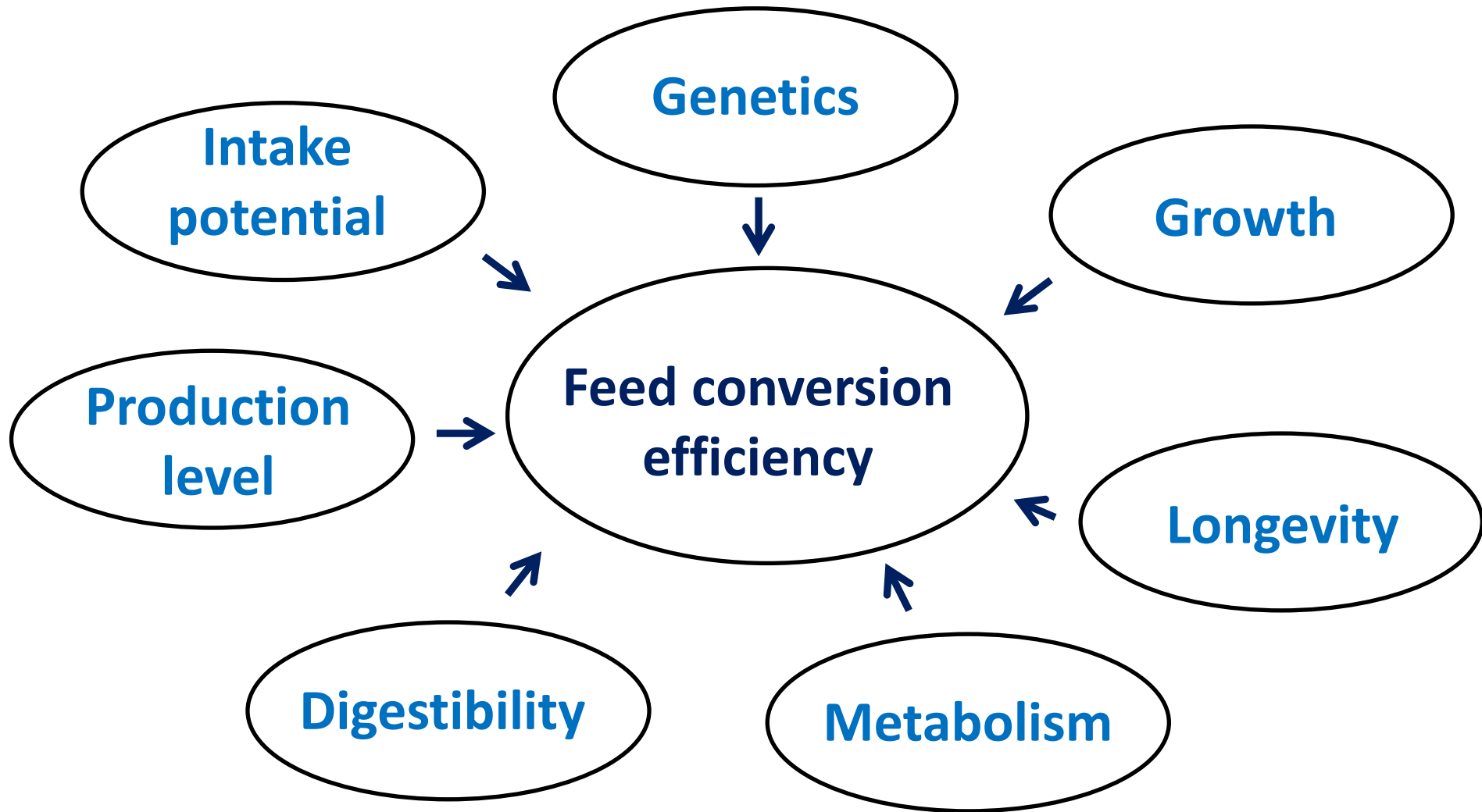
# Challenges to ruminant livestock production

- Economic
- Environmental
- Societal

## Scientific and technical solutions required

- Increase efficiency
- Lower emissions
- Improve product quality

# Technical challenge: Phenotyping of complex traits



# Advances in technologies: New phenotypes

## Technologies

- Sequencing/high density chips
- New “precision” devices for monitoring on farm
- FTIR and MS/MS: high throughput analysis

## Tools

- Characterise microbial populations
- More extensive phenotypes
- Genomic selection

# **New phenotypes for new breeding goals in dairy cattle**

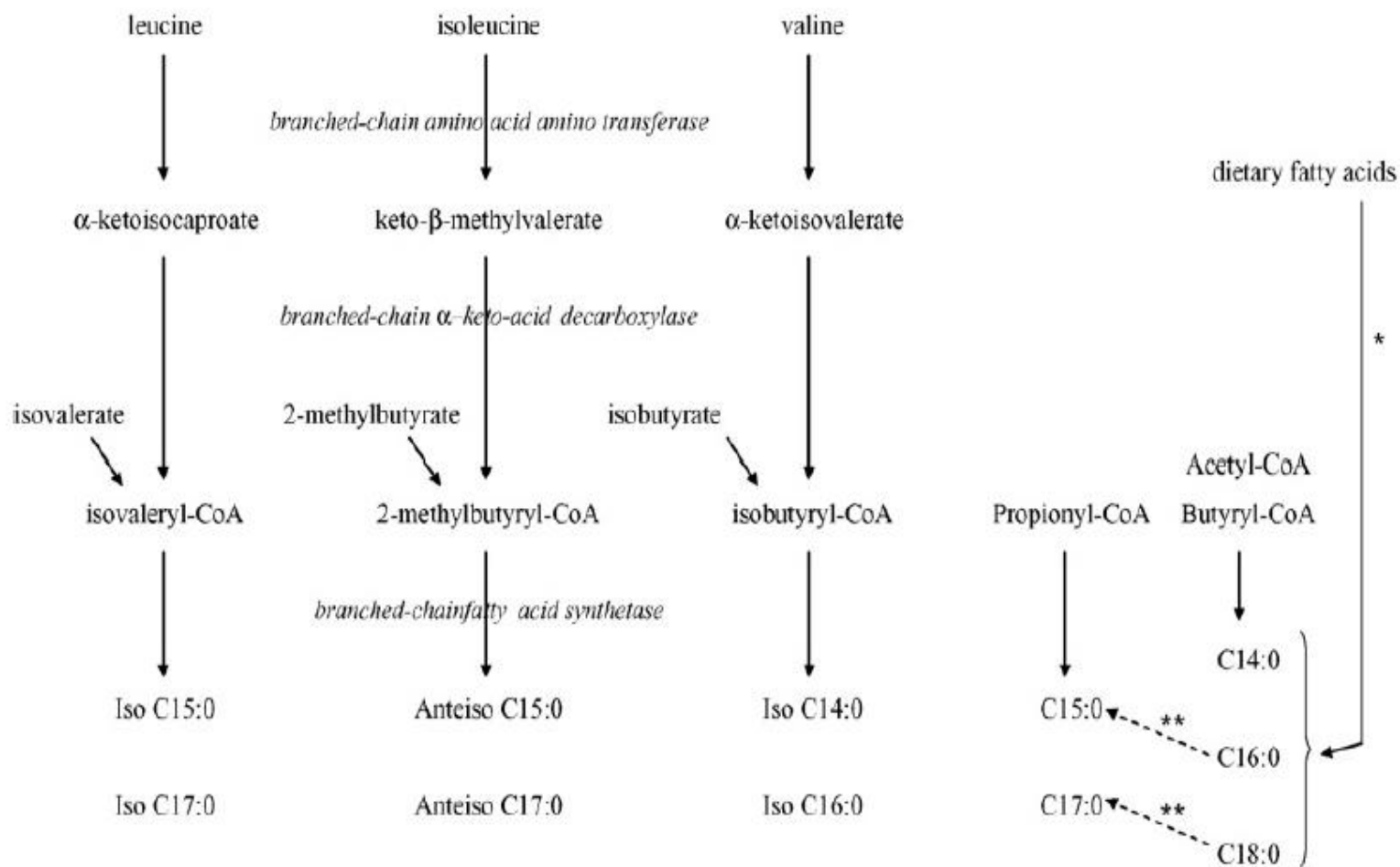
- **Large scale phenotyping: number of animals, number of traits and scales from molecule to whole animal**
- **Genetic selection requires phenotyping of thousands of animals that remains a major constraint**
- **Development of high-throughput methodologies are required for application on large populations according to standardized definitions and methods**

# Diverse range of potential biomarkers:

e.g. ruminal methanogenesis

- **Breath**
  - Gas concentrations
- **Rumen samples**
  - qPCR 16S gene
  - Archaeol
- **Milk**
  - Fatty acid composition
- **Faeces**
  - Archaeol

# Odd and branched chain fatty acid synthesis in ruminal bacteria





# Proportions of odd and branched chain fatty acids in bacterial membranes differ between species

	Fermentation products <sup>d</sup>	<i>Anteiso</i> C13:0	<i>Anteiso</i> C15:0	<i>Anteiso</i> C17:0	<i>Iso</i> C13:0	<i>Iso</i> C15:0	<i>Iso</i> C17:0	<i>Iso</i> C14:0	<i>Iso</i> C16:0	C13:0	C15:0	C17:0	C17:1
<i>R. albus</i> <sup>a</sup>	A	-	9.4	1.3	-	-	0.7	<u>20.6</u>	11.0	-	10.3	1.4	-
<i>B. fibrisolvens</i> <sup>a</sup>	A, B, F	6.4	<u>16.2</u>	8.6	6.8	10.4	5.7	10.8	11.1	2.9	7.8	4.3	3.5
<i>R. flavefaciens</i> <sup>a</sup>	A, S	-	2.3	2.9	-	<u>35.7</u>	5.2	2.5	7.3	0.1	3.2	0.5	-
<i>S. amylolytica</i> <sup>b</sup>	A, P												
N6		-	-	-	-	<u>52.6</u>	10.8	1.6	5.3	1.6	5.0	-	-
B24		-	-	-	-	0.1	0.3	-	0.6	1.4	<u>3.3</u>	1.3	0.6
<i>Prevotella</i> <sup>b,c</sup>	A, S	1.2	<u>36.7</u>	4.2	3.0	14.7	2.3	3.3	3.0	1.2	12.1	2.1	-
<i>L. multiparus</i> <sup>b,c</sup>	A, L, F	-	<u>4.0</u>	2.6	-	1.1	1.1	1.2	1.8	0.3	2.9	0.8	0.1
<i>S. dextrinosolvens</i> <sup>c</sup>	A, S	0.8	3.6	1.0	-	0.1	-	0.6	1.5	0.5	<u>4.0</u>	0.7	-
<i>R. amylophilus</i> <sup>b</sup>	A, S, F	-	<u>1.1</u>	-	-	-	-	-	-	0.5	<u>1.1</u>	0.3	0.1
<i>F. succinogenes</i> <sup>a</sup>	A, S	3.9	7.7	1.2	-	0.1	0.2	3.6	3.4	9.0	<u>30.2</u>	2.1	-
<i>S. bovis</i> <sup>b</sup>	L	-	0.9	-	-	-	-	0.4	0.2	0.6	<u>1.7</u>	1.2	0.2
<i>M. elsdenii</i> <sup>c</sup>	A, P, B	-	2.8	-	0.1	0.2	0.2	1.5	0.5	1.5	<u>6.0</u>	4.5	3.0
<i>E. ruminantium</i> <sup>b</sup>	B, L, F												
B1C23		-	-	-	-	17.7	1.4	-	-	5.4	<u>49.0</u>	1.5	-
GA195		-	<u>30.1</u>	1.7	-	0.4	0.2	6.1	3.7	0.4	6.5	0.4	-
<i>S. ruminantium</i> <sup>b</sup>	A, P, L	-	0.1	-	-	0.2	-	0.3	0.1	1.3	<u>6.0</u>	2.9	2.6

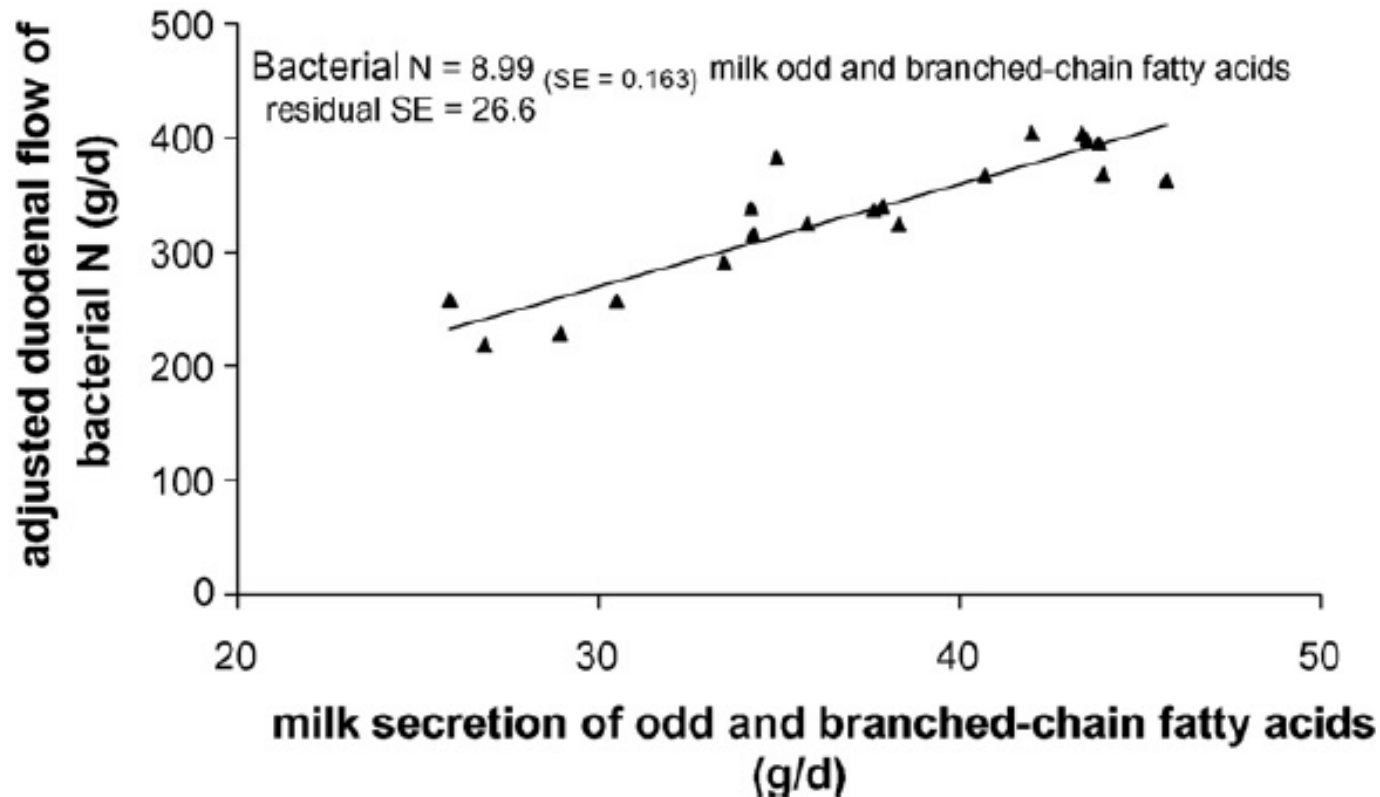
<sup>a</sup> Bacteria fermenting cellulose and hemicellulose.

<sup>b</sup> Bacteria fermenting starch.

<sup>c</sup> Bacteria fermenting sugar and pectin.

<sup>d</sup> A: acetate; S: succinate; B: butyrate; F: formate; P: propionate; L: lactate.

# Secretion of OBCFA in milk as a biomarker of microbial N at the duodenum

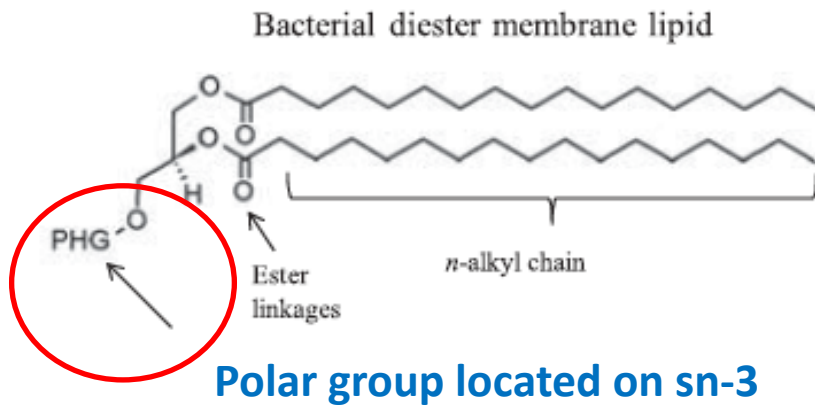


# Milk fatty acid composition as a biomarker of methane production

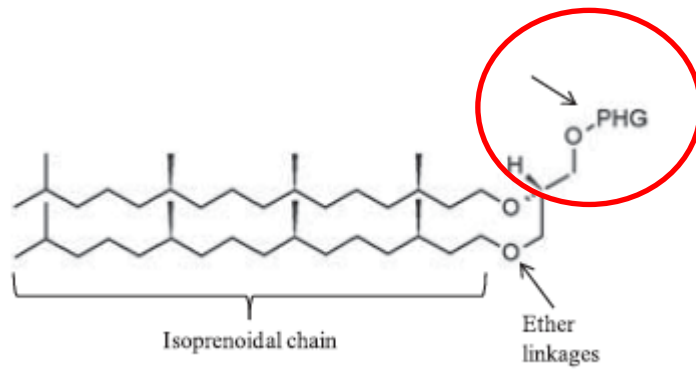
Reference	Number of observations (number of studies)	Method for measuring/ estimating methane production	Treatments	Percentage of variance in methane production explained by best combination of milk fatty acids	Individual milk fatty acids related to methane production:	
					Positive relationship	Negative relationship
Chilliard <i>et al.</i> (2009)	32 (1)	SF <sub>6</sub> tracer technique	Maize/grass silage and concentrates with different linseed products	95 (within study)	4:0 6:0 8:0 9:0 10:0 10:1 11:0 12:0 12:1 14:0 15:0 17:0 20:4	18:1 <i>trans</i> -16 + <i>cis</i> -14 18:2 <i>cis</i> -9, <i>trans</i> -13 16:1 <i>trans</i> -11 18:1 <i>trans</i> -12 18:1 <i>cis</i> -13 18:1 <i>trans</i> -13 + 14 18:1 <i>trans</i> -6,7,8 18:1 <i>cis</i> -15 + <i>trans</i> -17 18:2 <i>trans</i> -11, <i>cis</i> -15 18:1 <i>cis</i> -9 18:1 <i>cis</i> -10 18:1 <i>trans</i> -10
Mohammed <i>et al.</i> (2011)	16 (1)	Respiration chambers	Barley silage-based TMR with different crushed oilseeds	83 (within study)	8:0 Iso-16:0*	17:1 <i>cis</i> -9 18:1 <i>cis</i> -11 18:1 <i>cis</i> -13 18:1 <i>trans</i> -6,7,8 18:2 Iso-17:0/16:1 <i>trans</i> -6,7,8 18:2 <i>cis</i> -9, <i>trans</i> -13/ <i>trans</i> -8, <i>cis</i> -12 18:3
Dijkstra <i>et al.</i> (2011)	50 (10)	Respiration chambers	TMR based on grass and maize silages with a range of supplements (fumarate, diallyldisulphide, yucca powder, fatty acids, linseed products)	73 (within study)	Iso-14:0 Iso-15:0 Anteiso-7:0	17:1 <i>cis</i> -9 18:1 <i>trans</i> -10 + 11 18:1 <i>cis</i> -11
Casto Montoya <i>et al.</i> (2011) (only considered odd- and branched-chain fatty acids)	224 (13)	Calculation based on volatile fatty acid proportions	Wide range of forages and forage/concentrate ratios	66 (cross-validation)	Iso-14:0 Iso-15:0 Iso-16:0	15:0 17:0 + 17:1 <i>cis</i> -9

# Structural differences in membrane lipid of rumen archaea and bacteria

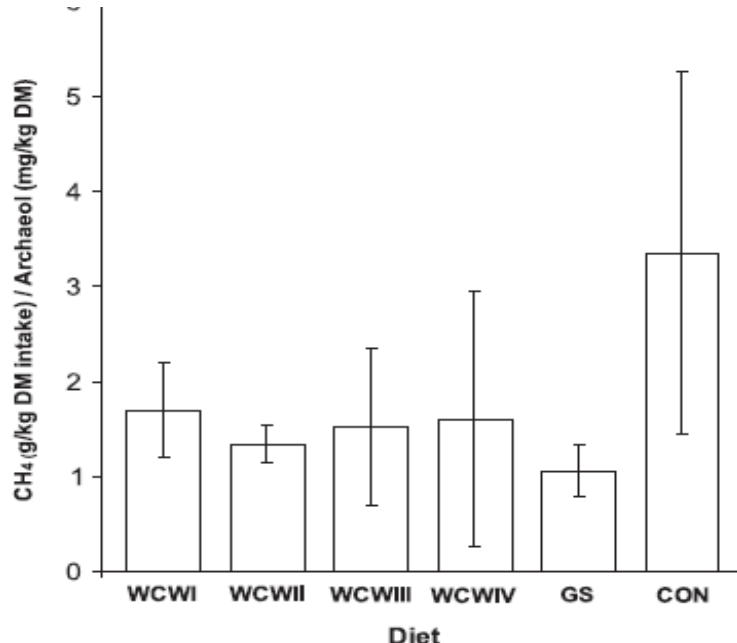
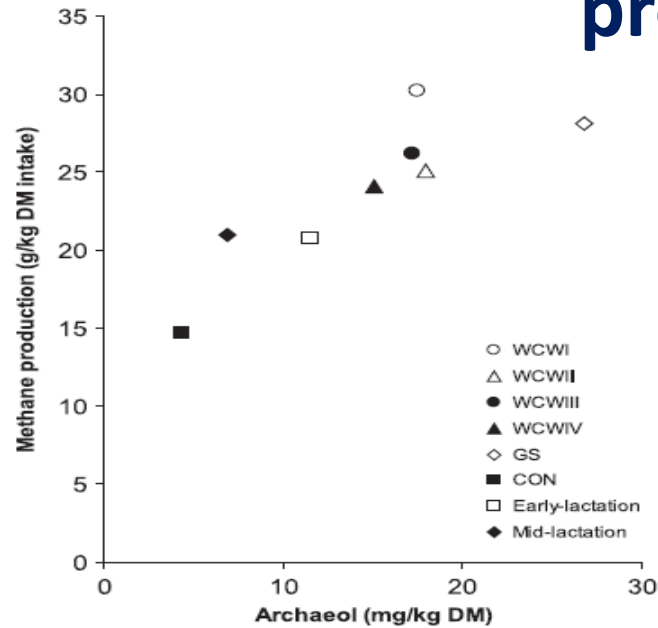
## Bacteria



## Archaea



# Faecal archaeol as a biomarker of methane production



- Easy sample collection and processing
- Close relationship for treatment means
- Considerable variation between animals
- Poor predictor of rumen methanogenesis-selective retention in the rumen

## **Phenotypes of rumen function**

- **Methane production**
- **Nutrient digestibility**
- **Rumen fermentation**

**Development of new tools avoiding traditional constraints in hard to measure phenotypes**

- **qPCR of 16S and 18S genes in ruminal digesta**
- **Metagenomics**

# Archaeal abundance in *post-mortem* ruminal digesta may help predict methane emissions from beef cattle

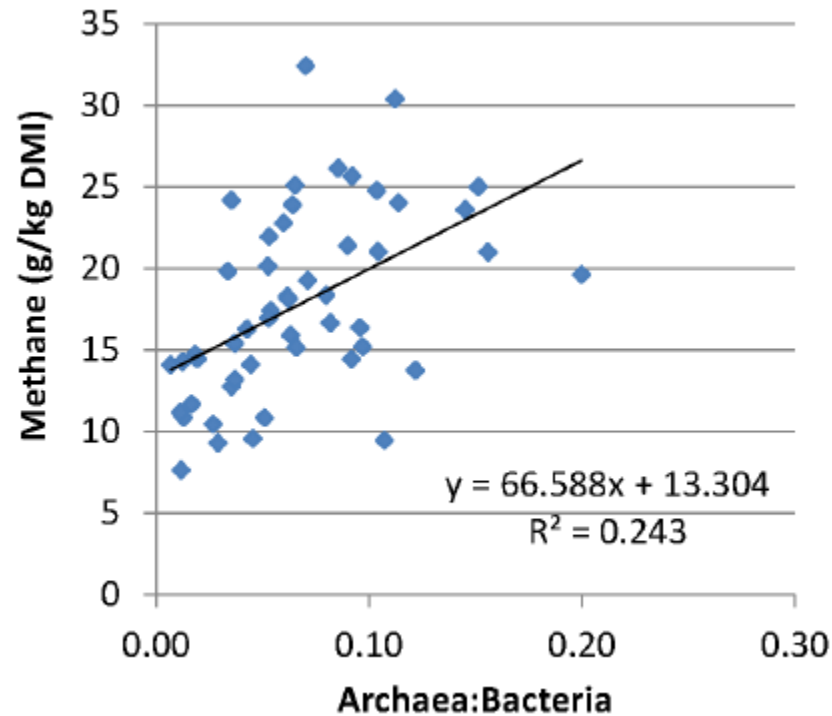


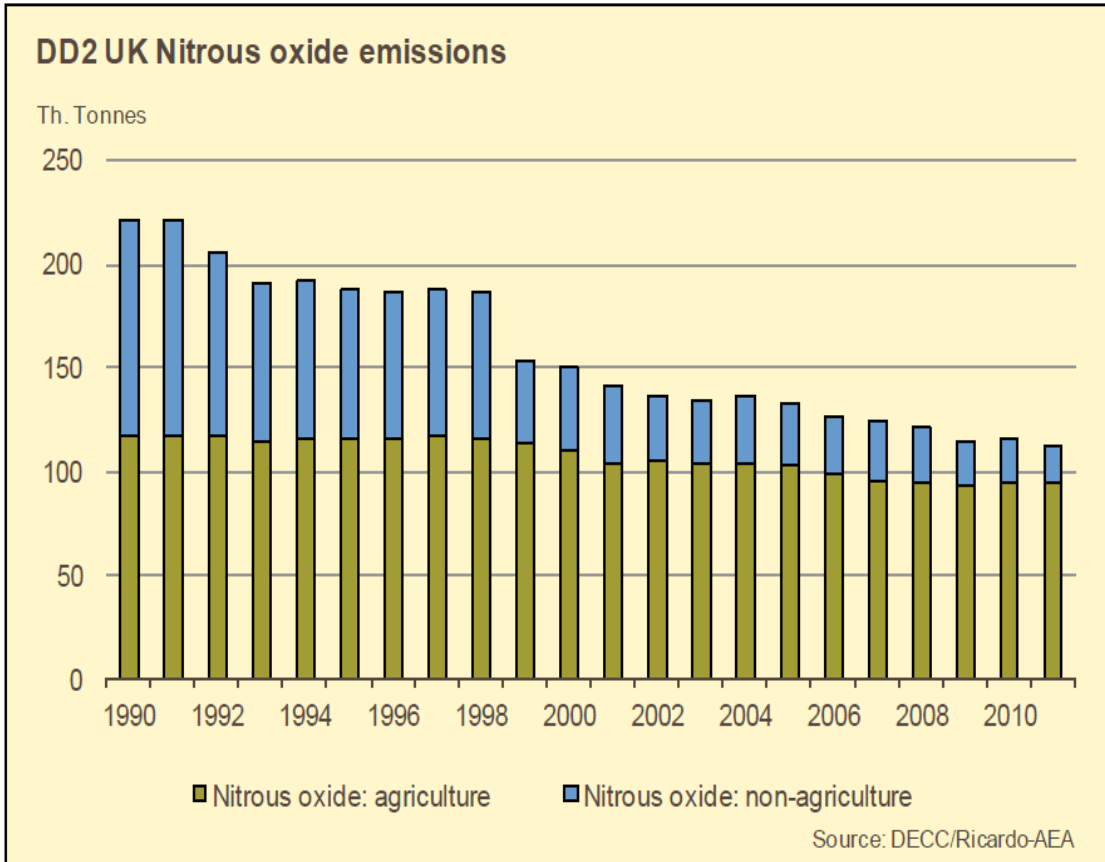
Figure 7 | Methane emissions and the archaea : bacteria ratio (A : B) in ruminal digesta samples taken from live animals immediately after exiting the respiration chamber.

# Nitrogen economy of the lactating cow





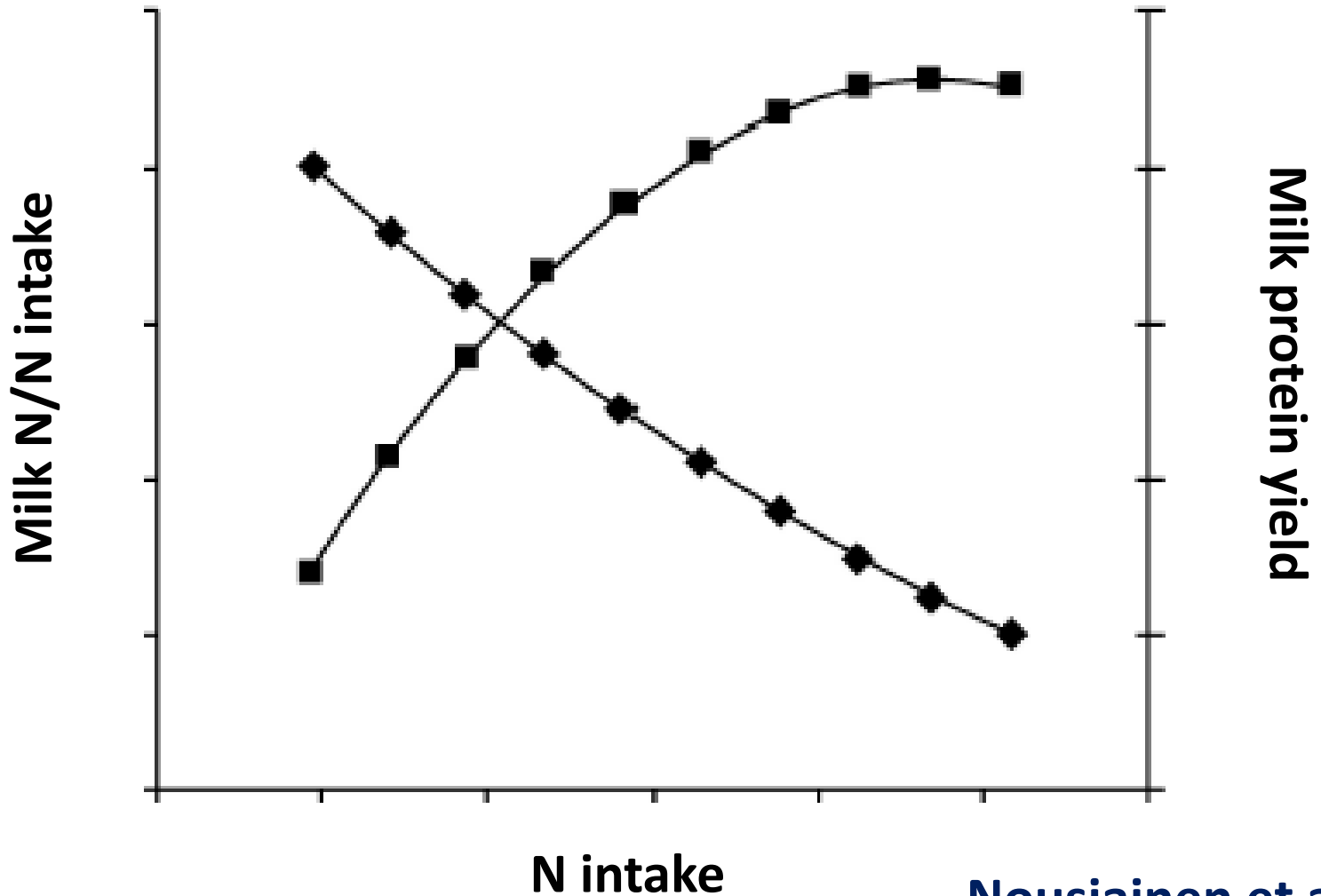
# Annual nitrous oxide emissions in the UK



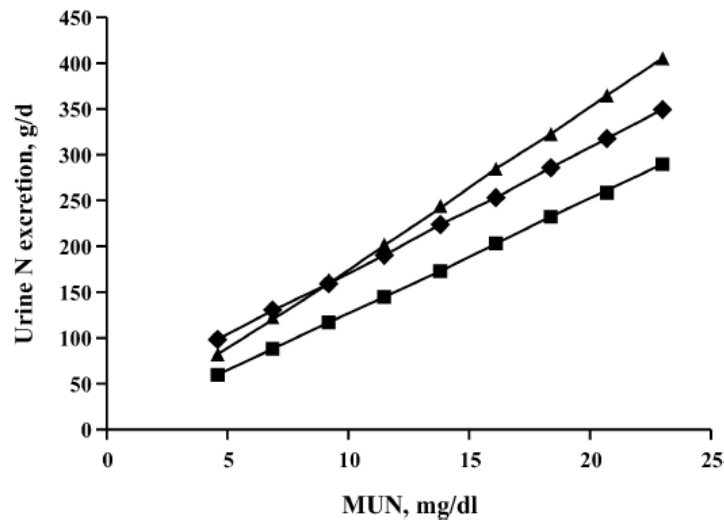
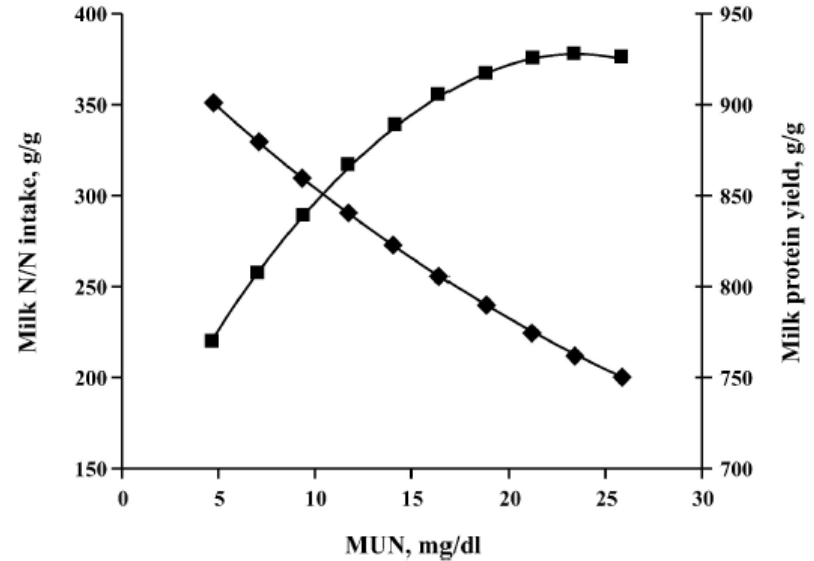
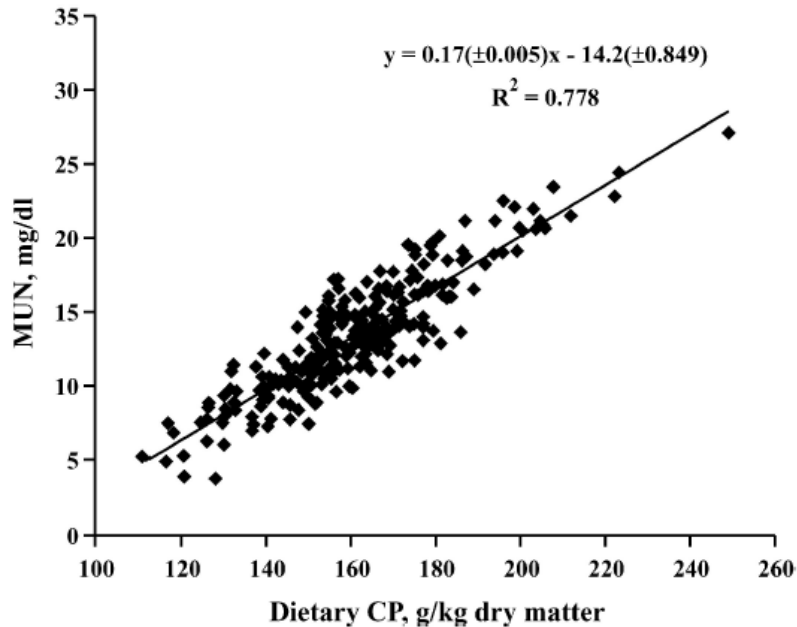
Total nitrous oxide emissions fell by 49% between 1990 and 2011. The largest reductions were in emissions from adipic acid production (a key raw material of polyurethanes) between 1998 and 1999. Reductions in industrial process emissions have continued to decline primarily due to decreases in the production of adipic and nitric acid.

- **N<sub>2</sub>O accounts for ca. 6% of UK anthropogenic greenhouse gas emissions**
- **About 80% of N<sub>2</sub>O from agriculture from soils**

# Nitrogen intake, production and nitrogen use efficiency



# Measurements of milk urea nitrogen



Meta-analysis based on 306  
treatment means from 50  
experiments

Nousiainen et al., 2004

## **Meta-analysis to understand between-animal variation in MUN and rumen ammonia N concentrations and the association with diet digestibility and N use efficiency**

- **1804 cow/period observations from 21 production trials**
- **450 cow/period observations from 29 metabolic studies**
- **Data were analyzed by mixed-model regression analysis**
- **Model included diet within experiment and period within experiment as random effects: effect of diet and period excluded**

# Results



- **Between cow variation in MUN 0.13 and 0.11 % for production and metabolic datasets**
- **Between cow variation in MNE 0.07 and 0.08 % for production and metabolic datasets**
- **Including MUN and RAN in the model accounted for more variation in MNE than milk yield alone**
- **Between-cow variation had a smaller influence on the relationship of MUN with urinary N excretion or MNE than when based on treatment means**

# Conclusions

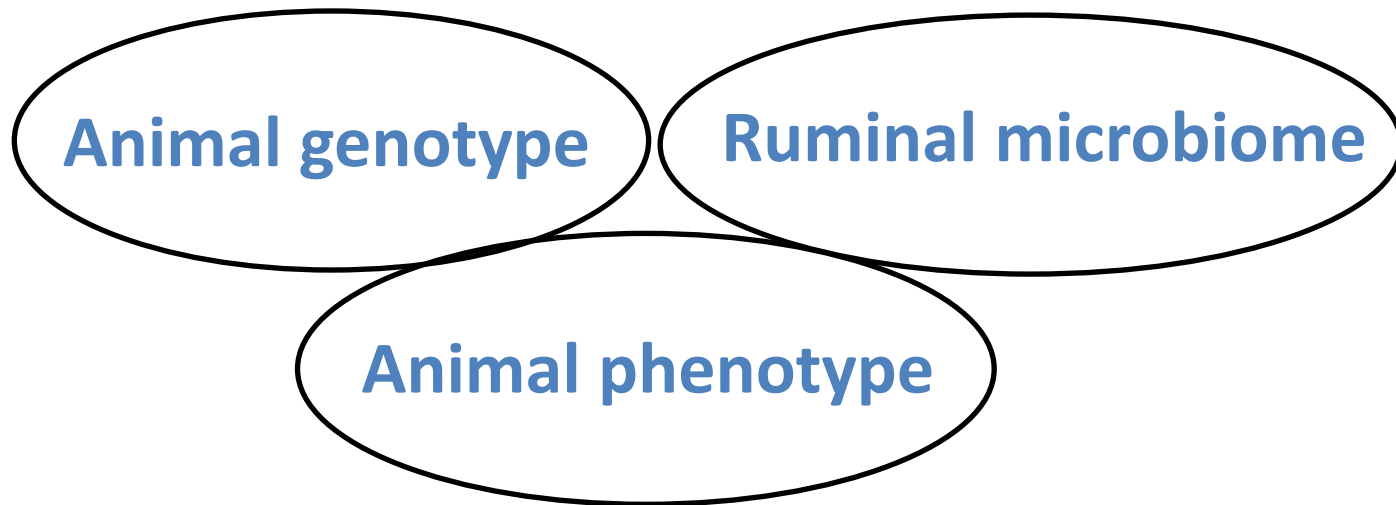


- **Between-cow variation in MUN had a smaller effect on MNE compared with published responses of MUN to dietary crude protein content**
- **Closer control over diet composition relative to requirements has greater potential to improve MNE and lower UN on farm than genetic selection**
- **Measurements of MUN are more useful as a management tool than as a phenotype for genetic selection of more nitrogen efficient cows**

# Future perspectives



## RuminOmics – Large scale data



**Intake, milk production, digestibility, methane output, fermentation characteristics, blood metabolome, milk fatty acid composition**



## **Project goals**

- **Understanding the role of host animal genetics, rumen microbiome and diet on methane production, nitrogen emissions, feed efficiency and milk quality**

## **Outcomes**

- **Generation of new large data for mining new biomarkers of rumen function, animal performance and milk fatty acid composition**



**Thank you for your attention**