

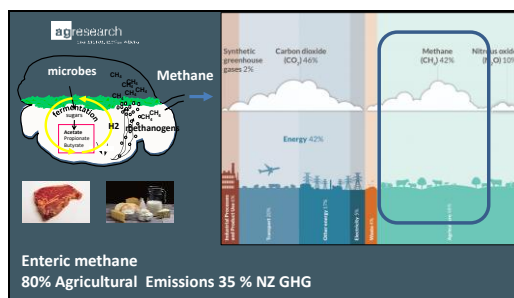
Slide 1

Using the microbiome as a proxy of methane, research and implementation, and future perspectives



Suzanne Rowe, Timothy Bilton, Melanie Hess, Setegn Worku & John McEwan

Slide 2



Slide 3

Mitigation Technologies for grazing livestock




Targets 10% by 2030 24-47% by 2050
mix of charges and incentives

Slide 4

Breeding for low methane

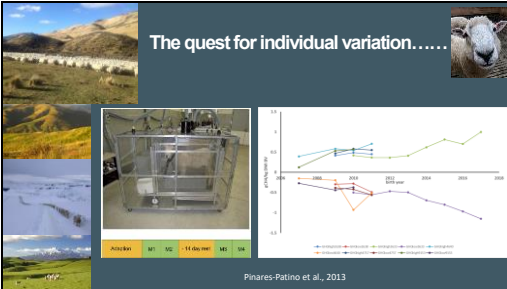
- Selection Lines in Sheep
- Genetic basis
 - Biology
 - Consequences of change
- Statistical prediction at scale
- Implementation and impact



agresearch
ANIMAL RESEARCH

Slide 5

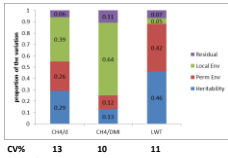
The quest for individual variation.....



Pinares-Patino et al., 2013

Slide 6

Heritability & repeatability



Group	CV%	Significance (h²)	Heritability	Repeatability	Farm Effect	Residual
CH4/ME	13	***	0.24	0.24	0.24	0.24
CH4/DM	10	***	0.15	0.15	0.15	0.15
CH4/ME	11	***	0.15	0.15	0.15	0.15

agresearch
ANIMAL RESEARCH

- Heritable and repeatable traits: benefit from repeated measurements
- Methane yield less variation and lower heritability and repeatability

Pinares-Patino et al 2013 Animal 7 Suppl 2:316-21

Slide 7



Slide 8

Low methane sheep

- ~12% less methane per kg feed eaten
- Increased rumen surface area
- Eats more, eats little and often
- Different microbial profiles
- Different energy profile – more propionate
- More lean growth, less fat, more wool
- Healthy and profitable

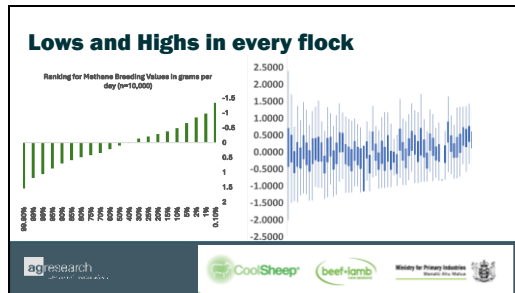
Bacteria
Fungi

Acetic acid (2C) Propionic acid (3C) Butyric acid (4C)

Methane production (g/day)

agresearch
Kitelmann et al., 2014

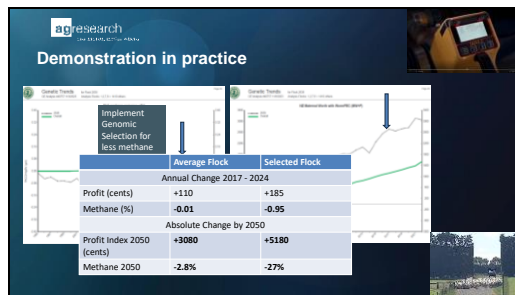
Slide 9



Slide 10



Slide 11





Slide 12

Slide 13

Breeding for low methane

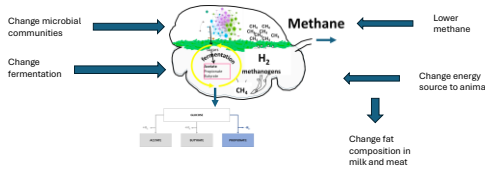
- Technical measurement
- Genetic basis
 - Biology
 - Consequences of change
- Statistical prediction at scale
- Implementation and impact





Slide 14

Breeding for low methane



Change microbial communities


Change fermentation

Methane

Lower methane


Change energy source to animal


Change fat composition in milk and meat



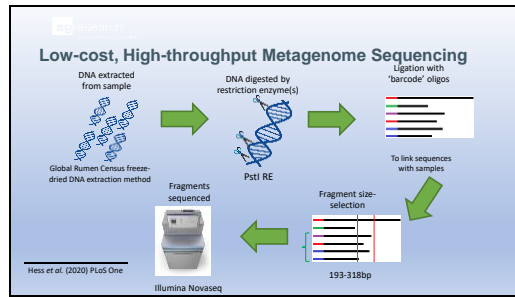
Slide 15

Live Sampling

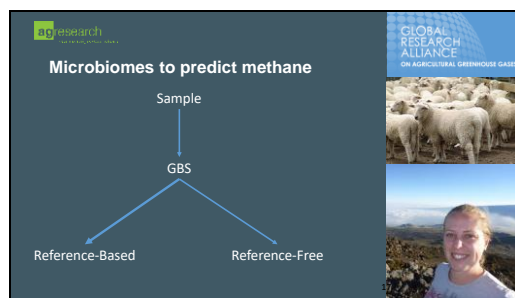




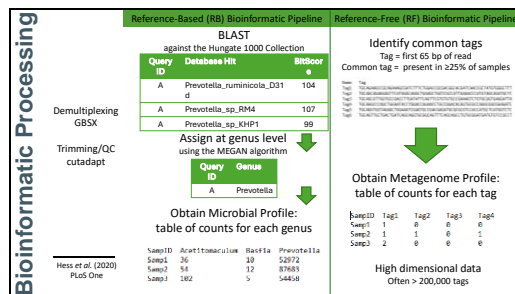
Slide 16



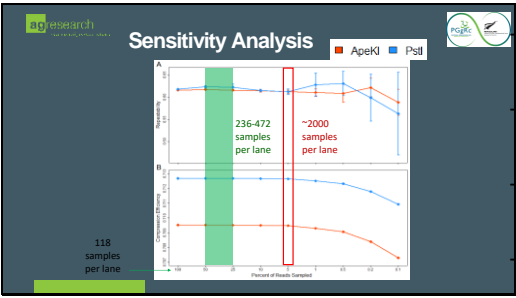
Slide 17



Slide 18



Slide 19



Slide 20

agresearch
ANIMAL GENOMICS RESEARCH

Cattle sequencing results compared to sheep

Information	Parameter	Sheep1	Sheep2	Cattle
Sequencing	Number of Samples	236	654	186
	Samples per Lane	118	164	188
	Number of Reads/Sample	2.7M ± 680k	1.5M ± 586k	759k ± 147k
Reference-Based	Percent Assigned	6.8 ± 1.8	6.3 ± 1.2	9.3 ± 1.6
Reference-Free	Number of Tags*	503k	375k	423k
	Percent Assigned	38.1 ± 16.2	39.1 ± 3.2	64.3 ± 6.8

* Tags are 65bp reads present in at least 25% of samples

8

Slide 21

agresearch
ANIMAL GENOMICS RESEARCH

Comparison of Approaches

Correspondence Analysis

Pedigree-based estimates for the first dimension

Approach	h^2	Repeatability	Correlation CH_4 Yield
16S	0.26 (0.23)	0.45 (0.08)	0.63 (0.49)
ApeKI_RB	0.58 (0.32)	0.61 (0.06)	0.63 (0.31)
PstI_RB	NE	0.60 (0.06)	NE
ApeKI_RF	0.18 (0.25)	0.60 (0.06)	NE
PstI_RF	0.24 (0.27)	0.62 (0.06)	0.88 (0.25)

* Permanent Environment constrained to zero

Hess et al. (2019)

7

Slide 22

Trait prediction from rumen microbial profile

- Use the rumen microbial profile as proxy trait for
 - Methane emissions
 - Feed efficiency

Bioinformatics:

- Reference-free pipelines (Hess et al., 2020)
- Tags: Unique 65 bp reads
- Present in 25% of samples
- Matrix of tag counts

Sample	Tag1	Tag2
Samp1	1	0
Samp2	0	0
Samp3	2	1

Microbial Relationship Matrix (MRM):

- Log transformed counts
- Standardized by cohort
- Correlation standardized counts (Hess et al., 2020)

	Samp1	Samp2	Samp3
Samp1	1.00	0.98	0.57
Samp2	0.98	1.00	0.64
Samp3	0.57	0.63	1.00

Modelling...

agresearch

Slide 23

Fitted methane

Linear Mixed Models:

Model 1 (Genomics)

$$y_i = \mu + f_i + a_i + e_i$$

Model 2 (RMHC)

$$y_i = \mu + f_i + m_i + e_i$$

Model 3 (Genomics + RMHC)

$$y_i = \mu + f_i + a_i + m_i + e_i$$

$a_i \sim N(0, \sigma_a^2 G)$, G = GRM
 $m_i \sim N(0, \sigma_m^2 M)$, M = MRM
 $e_i \sim N(0, \sigma_e^2 I)$, I = identity
 m_i = microbial value

Predicted Methane

- Predicted breeding values \hat{a}_i
- Predicted microbial values \hat{m}_i

Prediction Accuracy

- Correlation between y_i^* and \hat{a}_i or \hat{m}_i

Adjusted phenotypes (y_i^*)

- Methane adjusted for fixed effects
- $y_i^* = \mu + e_i$

y_i = Methane or Methane Yield

f_i = fixed effects:

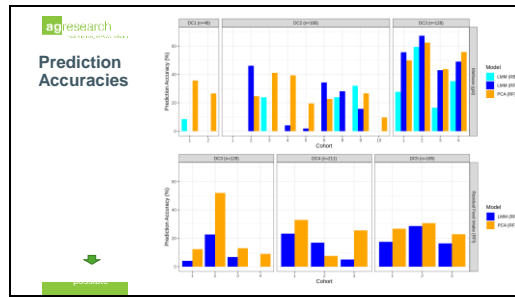
- Ages of dam
- Birth rear rank
- Birth day deviation
- From flock mean
- Contemporary group
- Flock*YOB*Sex

agresearch

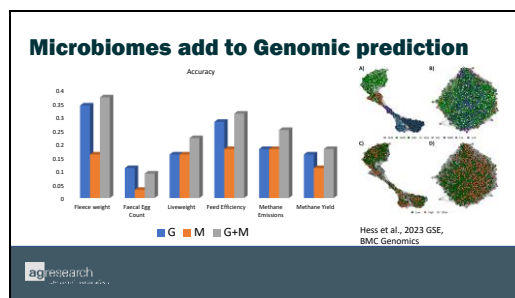
Slide 24

	Linear Mixed Model	Dimension Reduction using PCA
Rumen microbial profile	Microbial relationship matrix (MRM) Correlation of log ₁₀ (x+1) microbial proportions Normalized within cohort (Hess et al. (2020) PLoS One)	Principal component analysis (PCA) Centered log-ratio transformed microbial counts Pseudo counts of 1 RF profile
Adjusted phenotypes	$y_i = \mu + X_i \beta_i + y_i^*$ y_i^* = residual term	$y_i = \mu + X_i \beta_i + y_i^*$ y_i^* = residual term
Modelling	$y_i = \mu + X_i \beta_i + m_i + e_i$ $m_i \sim N(0, \sigma_m^2 M)$ m_i = microbial breeding value M = MRM	$y_i^* = \mu + PC_1 + \dots + PC_m + e_i$ $PC_j = j^{th}$ principal component
Prediction accuracy	Correlation between y_i^* and \hat{m}_i \hat{m}_i = predicted microbial values	Correlation between y_i^* and \hat{y}_i^* \hat{y}_i^* = predicted adjusted phenotypes

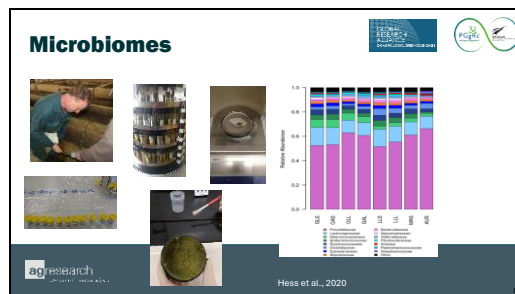
Slide 25



Slide 26



Slide 27



Slide 28

Microbiomes for prediction

- Developed protocols sampling and preserving
- Optimised pipelines
- Field testing in sheep and cattle
- Used gastric tubing and oral swabs

Slide 29

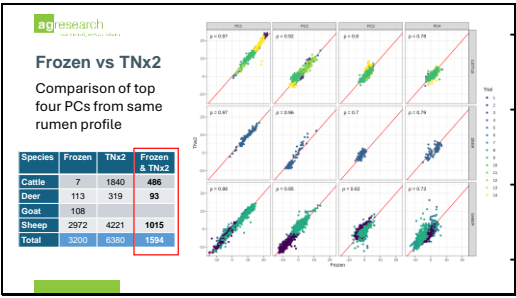
Workflow for processing rumen samples

Slide 30

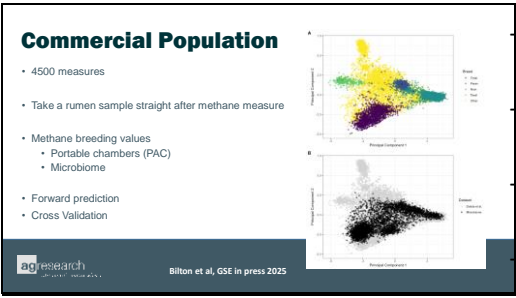
Microbial Sampling

Bilton GGAA talk

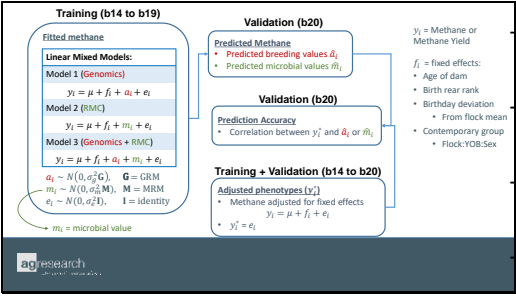
Slide 31



Slide 32



Slide 33



Slide 34

Results

Prediction accuracy of **breeding**/microbial values for animals in **validation** set

Trait	Model	r_{breed}	r_{microb}
Methane	1 (G)	0.32 ± 0.09	
	2 (M)		0.31 ± 0.06
	3 (G + M)	0.30 ± 0.11	0.31 ± 0.06
Methane Yield	1 (G)	0.34 ± 0.09	
	2 (M)		0.28 ± 0.06
	3 (G + M)	0.34 ± 0.12	0.29 ± 0.06

r_{breed} is the accuracy of the estimated **breeding values** computed as $(1-PEV/a_{\mu}^2)^{1/4}$
 r_{microb} is the accuracy of the estimated **microbial values** computed as $(1-PEV/a_{\mu}^2)^{1/4}$

agresearch

Slide 35

Bivariate Analysis

Parameter	Methane		Methane Yield	
	PAC	RMC	PAC	RMC
Heritability	0.39 ± 0.03	0.19 ± 0.07	0.31 ± 0.05	0.18 ± 0.05
Genetic correlation	0.76 ± 0.14		0.66 ± 0.13	
Phenotypic correlation	0.35 ± 0.03		0.32 ± 0.03	

Model:

$$[\hat{M}_i, y_i^*]' = \mu + a_i + e_i$$

- \hat{M}_i = scaled methane trait for validation animals predicted from RMC profiles using training set
- y_i^* = adjusted phenotype for validation animals
- $a_i \sim N(0, \sigma_a^2 G)$ G = GRM

agresearch

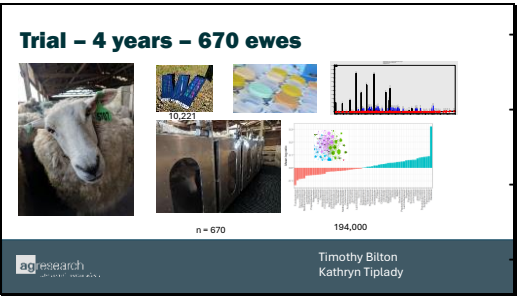
Slide 36

Other Phenotypes ?

- Methane
- Rapid methane
- Animal DNA
- Microbes
- Rumen outflow
- Milk and meat

agresearch

Slide 37



Slide 38

Phenotypes 670 ewes over 4 years

Proxy	Number of Variables
Host genomics (SNPs)	10,221
Rumen profiles (RMC)	193,233
Rumen Volatile fatty acids (VFA)	7
Milk Fatty Acids (FA)	32
Mid Infra Red spectra (MIR)	571

agresearch
LIFE SCIENCE RESEARCH

Bitton et al, 2025

Slide 39

Proxies


	PAC	Methane	RMC	Rumen VFA	Milk FA	MIR
PAC		0.24 ± 0.08	0.23 ± 0.05	0.16 ± 0.05	0.24 ± 0.05	0.18 ± 0.05
Methane			0.28 ± 0.08	0.57 ± 0.03	0.54 ± 0.04	0.25 ± 0.05
RMC				0.10 ± 0.07	0.36 ± 0.04	0.28 ± 0.05
Rumen VFA					0.32 ± 0.10	0.37 ± 0.05
Milk FA						0.23 ± 0.08
MIR						

Heritabilities on diagonal; genetic correlations below diagonal, phenotypic correlations above



agresearch
LIFE SCIENCE RESEARCH


Slide 40

Proxies for breeding					
	Methane Yield	Microbial Profiles	Rumen VFA	Milk Fatty acids	Milk herd test
Heritability	0.24 ± 0.08	0.28 ± 0.08	0.10 ± 0.07	0.32 ± 0.10	0.23 ± 0.08
Genetic correlation	1	0.76 ± 0.22	0.18 ± 0.34	0.57 ± 0.23	0.71 ± 0.23
Efficiency	1	80%	14%	61%	70%


 agresearch
advancing animal genetic research

Slide 41


Proxy measures – same spend			
		Number	Genetic Gain
	Methane (Respiration chamber)	1	0.5
	Rapid methane (PAC)	5	1
	Fatty acids	25	x1.8
	Microbial profiles	80	x1.8
	Milk herd test	1000	x2.1

 agresearch
advancing animal genetic research

Slide 42



- Requires a specific protocol
- Ranks animals well
- Fast
- Validation at Grazing

 agresearch
advancing animal genetic research

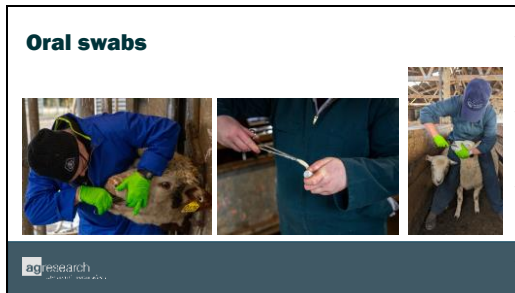
Slide 43



Slide 44



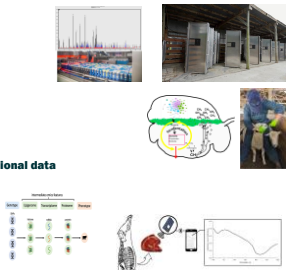
Slide 45



Slide 54

Next steps

- Predictors
 - Oral swabs – non invasive
 - Spectral profiles – low cost
- Multi-omics
- Interrogate structural & functional data
- Cross species and systems
- Global relevance




Slide 55

research
The Data Science Library

Turning points


- Portable chambers – new measurement technology for rapid measures
- Understanding diurnal profiles
- Sequencing microbiomes – information content
- Statistical normalisation of phenotypes – statistical robustness
- Preservative solution taking out dry ice/grinding step
- Expressing in units that fit calculators



Slide 56

Conclusions

- Breeding for low methane works in practice
- Biological signature in microbes and in host
- Proxies can double the rate of genetic gain for the same spend
- Requires development of commercial standardised testing
- Test global utility through collaboration



Slide 57

Acknowledgements

Funding

New Zealand Greenhouse Gas Centre
Pastoral Greenhouse Gas Research Consortium
New Zealand Government to support the Global Research Alliance on Agricultural Greenhouse Gases



Mapping the New Zealand Ruminant Landscape (C10X1807)





John McEwan
Melanie Hess
Hannah Henry
Timothy Bilton
Setegn Alemu



