



# Forages for Climate Mitigation

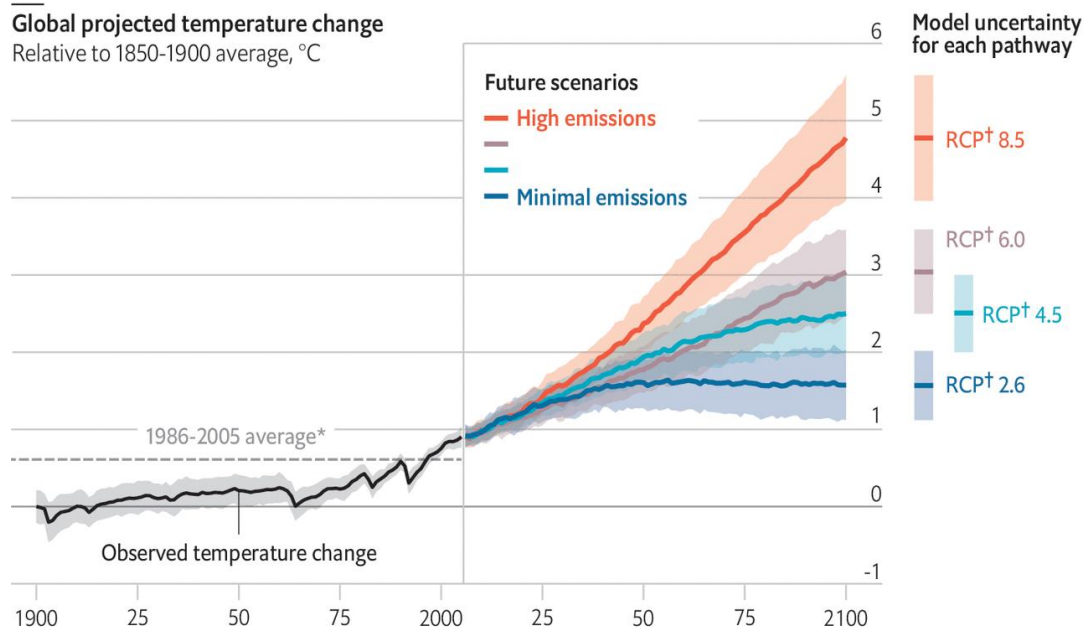
J Arango, C Arndt, N Bate, K Beauchemin, C Costa, JJ González, B Hatew, A Jaramillo, A Jarvis, C Jones, H Montgomery, A Muchugi, D Nkrumah, C Patiño, I Rao, B Rischkowsky, M Sánchez, A Tsivelikas, J Wamatu, P Wenzl, I Wright, M Yazbek

# Climate change and greenhouse gases

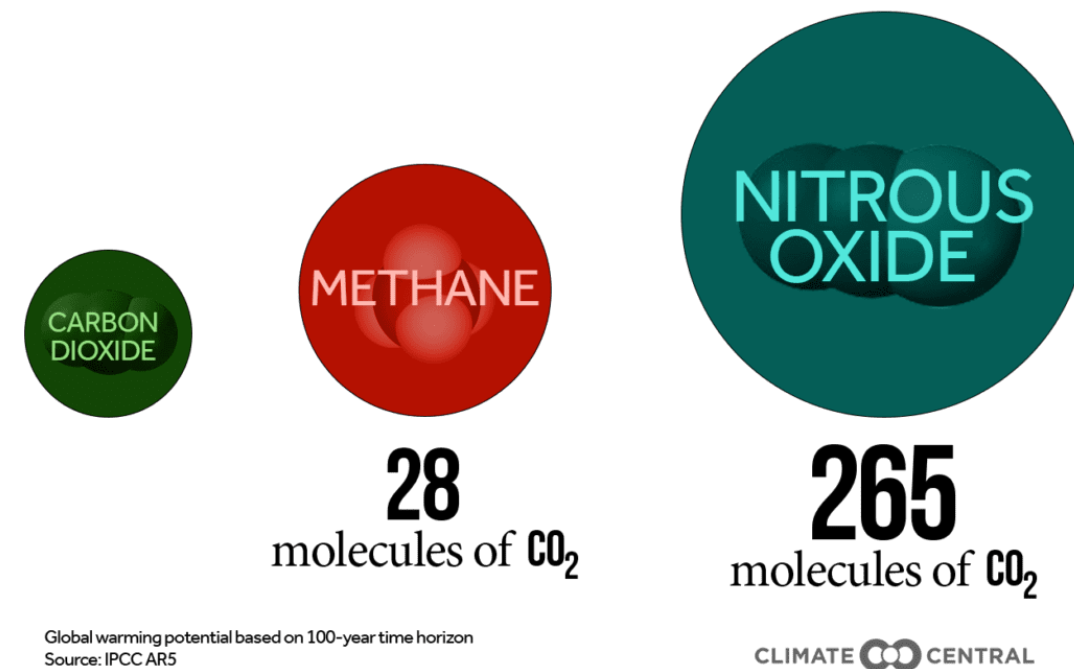
GHG emissions will result in warming of about **3.2 °C above pre-industrial levels** by 2100, leading to an unrecognizable world<sup>1</sup>.

CH<sub>4</sub> is 86x more potent than CO<sub>2</sub> (28x in 100 years): it accounts for **30% of global warming**<sup>2</sup>.

→ Climate models can guide policy even if they are not precise

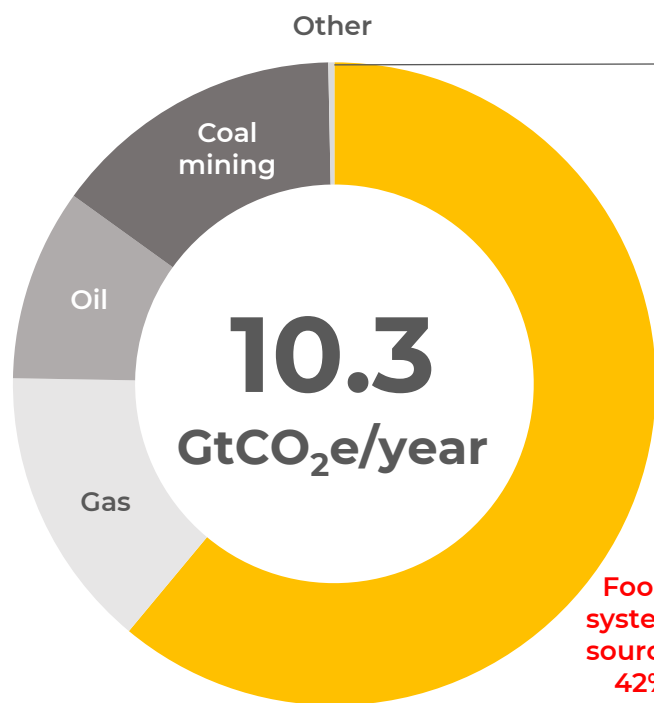


Source: IPCC AR5, adjusted to an 1850-1900 baseline \*Uncertainties calibrated to 1986-2005, as shown †Representative Concentration Pathway  
The Economist

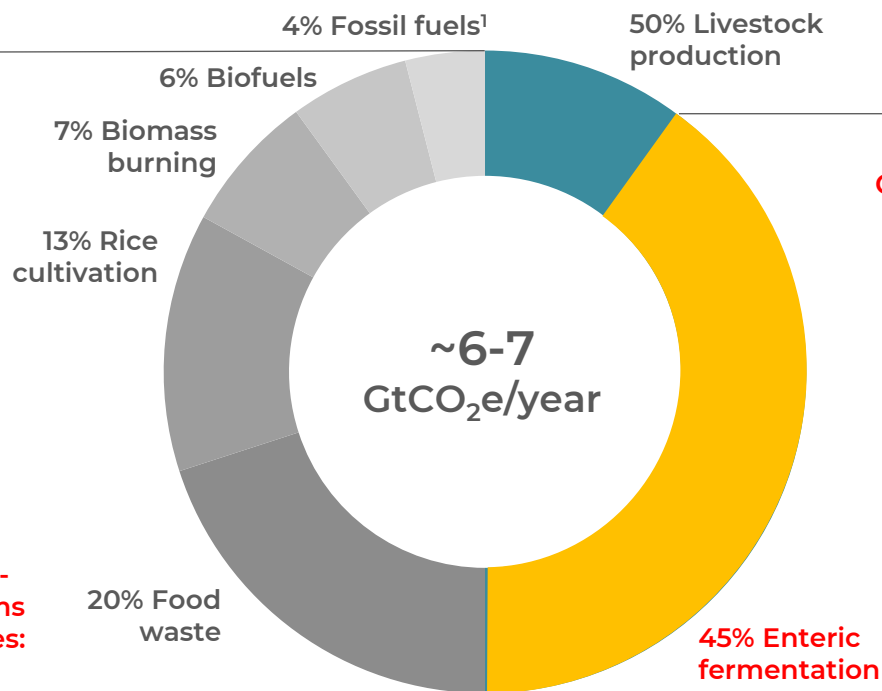


<sup>1</sup>IPCC, 2023; <sup>2</sup>Global Methane Hub

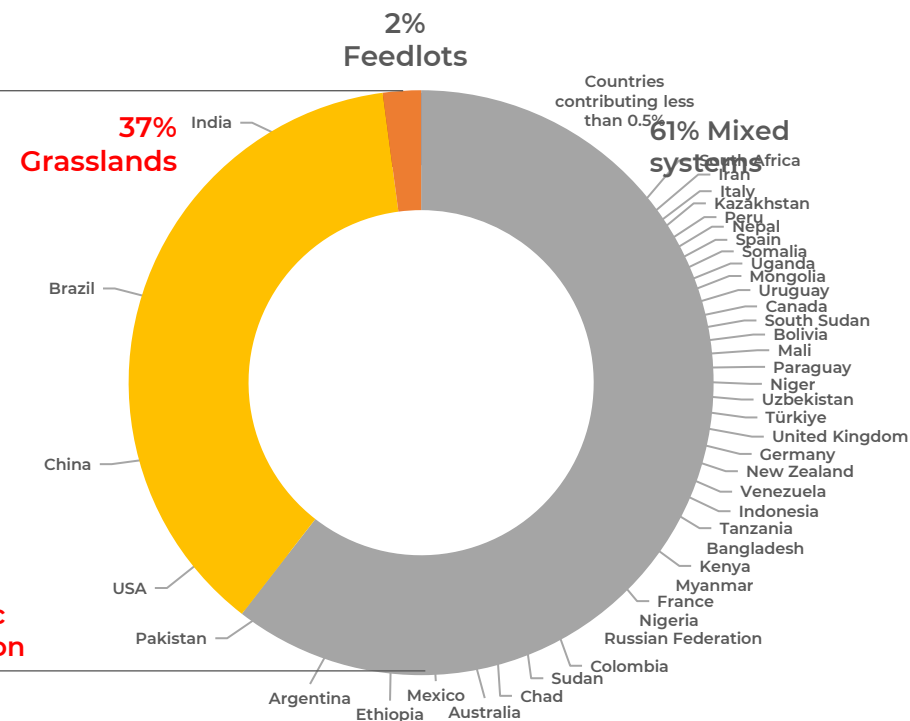
# ENTERIC METHANE CONTRIBUTION TO GLOBAL AND FOOD SYSTEM METHANE



Global anthropogenic methane emissions (2017)<sup>1</sup>



Food system methane emissions



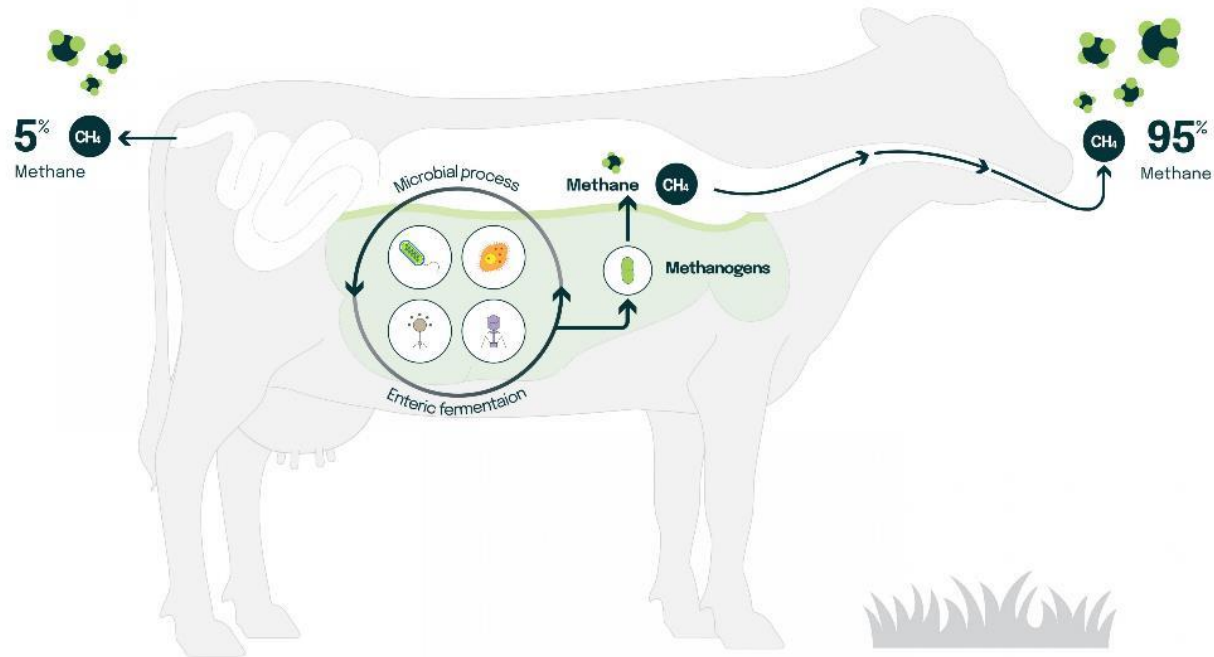
Proportions of enteric methane emissions

<sup>1</sup>Saunio et. al 2020: Total anthropogenic emissions are based on estimates of a full anthropogenic inventory and not on the sum of the "agriculture and waste", "fossil fuels", and "biofuel and biomass burning" categories due to methodology of adding different inventories. IPCC AR6 WGIII (2022). Available at: <https://www.ipcc.ch/report/ar6/>

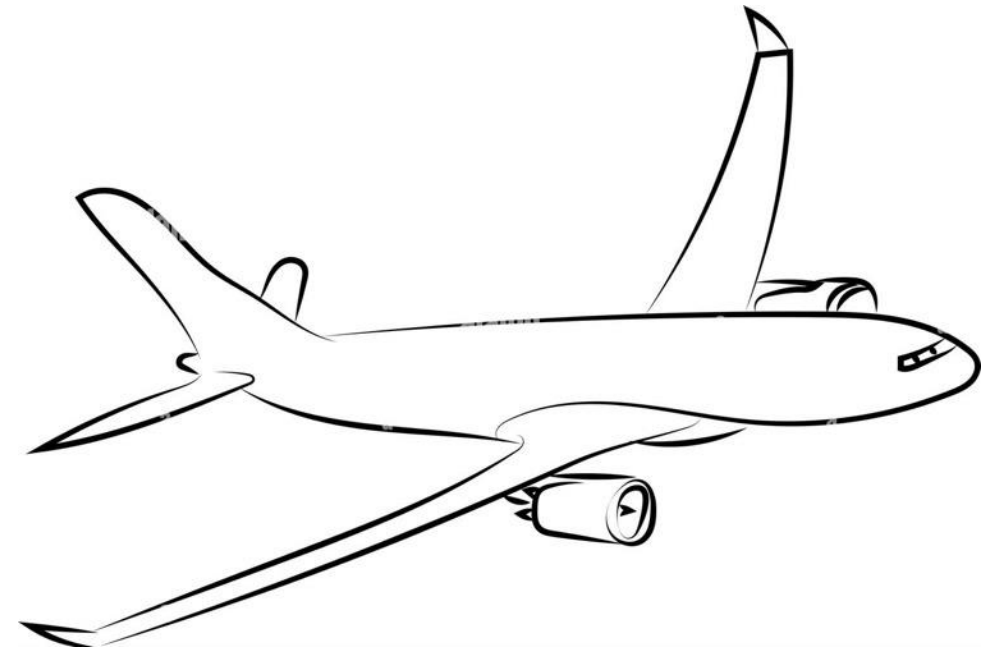
<sup>2</sup>Hegarty RS, Cortez Passetti RA, Dittmer KM, Wang Y, Shelton S, Emmet-Booth J, Wollenberg E, McAllister T, Leahy S, Beauchemin K, Gurwick N. 2021. An evaluation of emerging feed additives to reduce methane emissions from livestock. Edition 1. A report coordinated by Climate Change, Agriculture and Food Security (CCAFS) and the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) initiative of the Global Research Alliance (GRA).

# Context

- 30% of global warming due to  $\text{CH}_4$
- 42% of  $\text{CH}_4$  emissions: food systems
- 45% of food-system emissions: enteric  $\text{CH}_4$
- Enteric methane: 5.7% of global warming
- Project targets 2.1% of global warming (grasslands)



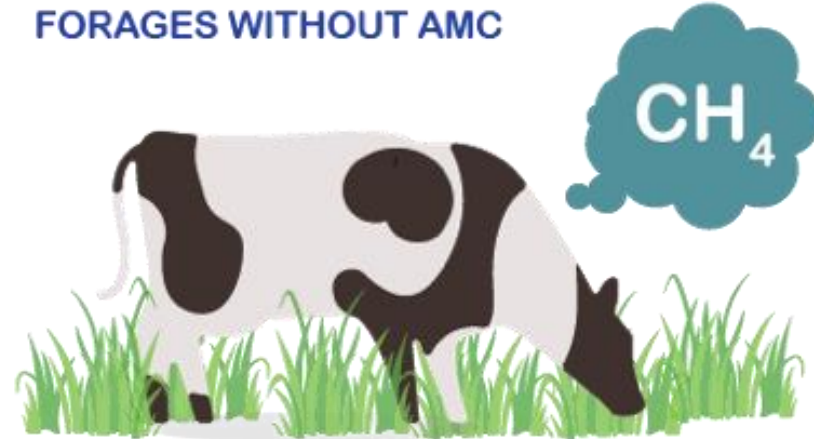
Aviation industry: 2.4 - 3.5% of global warming



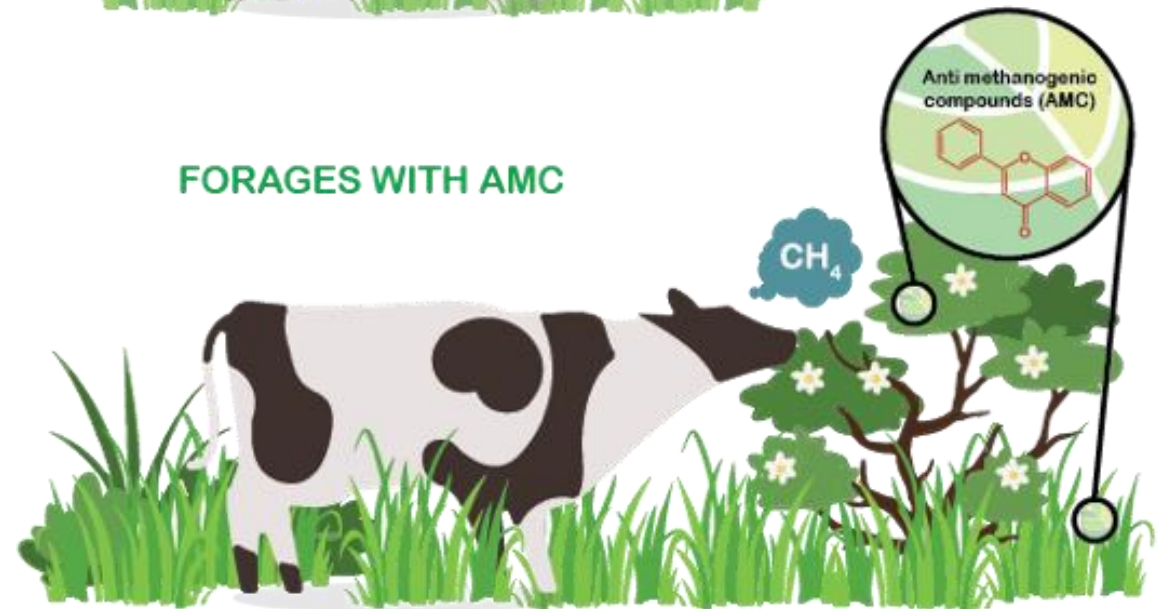
# Project goal

Identify, develop & deploy forages containing **Anti-Methanogenic Compounds (AMC)** in cattle production systems in the **Global South** to achieve an outsized reduction of greenhouse gas emissions per dollar invested, equivalent to taking 7 million internal combustion-engine cars off the road each year

FORAGES WITHOUT AMC

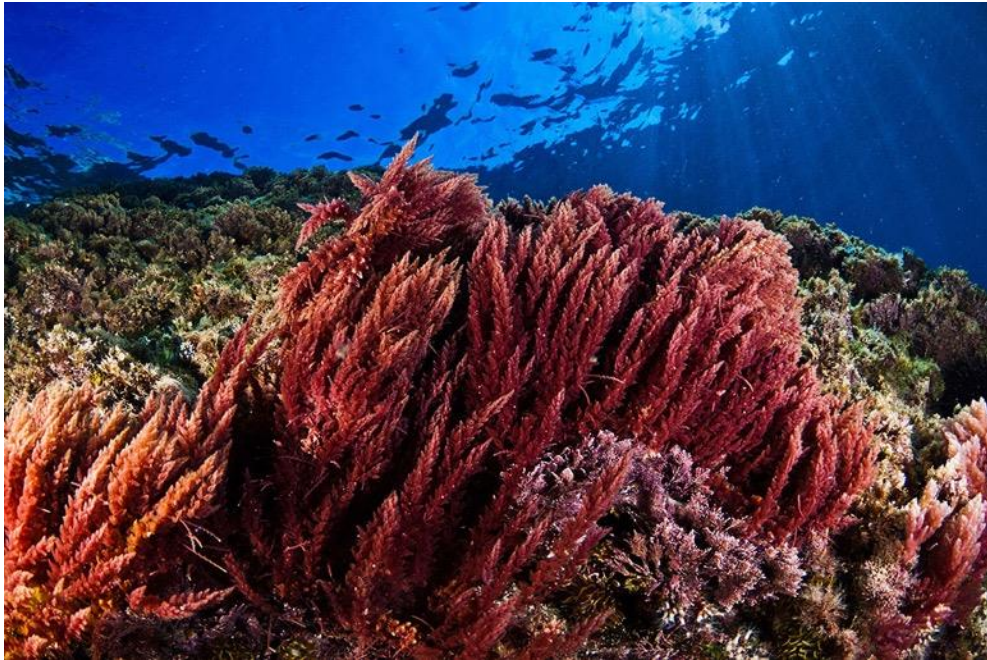


FORAGES WITH AMC





# AMC as feed additives



- Seaweed containing bromoform (BF) as a feed additive with anti-methanogenic properties
- Up to 90% reduction in methane production (beef on grain diet) but animal-production data are lacking
- But: animal/human health concerns
  - BF is a human carcinogen
  - BF residues have been detected in milk in some studies
- Expensive

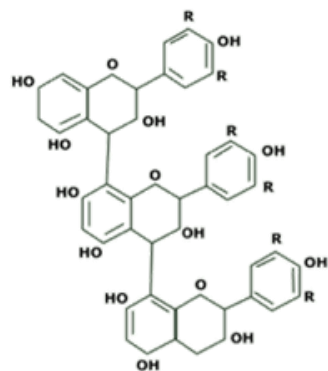
## Target Metabolites



*Leucaena  
leucocephala*

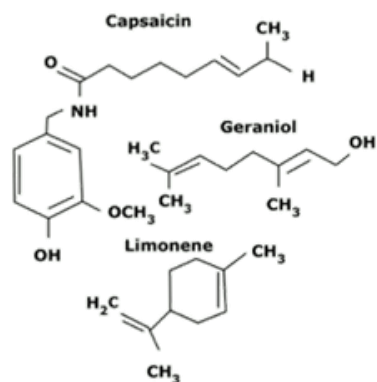
Plants rich in these  
secondary metabolites  
can be used as  
feedstock to reduce  
enteric CH<sub>4</sub> production

## Tannins



- Protozoo-methanogen interaction
- Limits nutrient availability

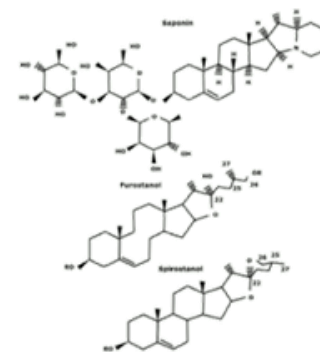
## Essential Oils



- Disruption of Cytoplasmic membrane
- Effect dependent on the EO evaluated

- More studies required

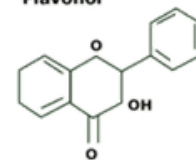
## Saponins



- Disrupt protozoal membrane: sterols-saponin complex
- Influence dynamics of Ca<sup>2+</sup> channels and N<sup>+</sup>-K<sup>+</sup> ATPases.

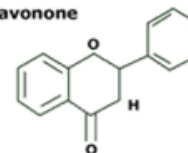
## Flavonoids

Flavonol



- Inhibits Cytoplasmic membrane, synthesis of the bacterial cell wall.

Flavonone



- Increase in the production of propionate relative to acetate

# CGIAR forage collections



The Alliance, ICARDA & ILRI collectively conserve almost 71,000 accessions used as forages, most of them collected/used in the Global South



# Previous studies

## Secondary metabolites in a wide variety of plants may mitigate CH<sub>4</sub> emissions

**TABLE 1.** Effect of plant species or plant extracts containing secondary metabolites on enteric methane mitigation in ruminants as measured in open-circuit respiration chambers in tropical regions.

References	Scientific name	Family	Part of plant	Tannins	Saponins	Essential oils	Flavonoids	Methane mitigation (%) over control ration
<b>Secondary compounds</b>								
(44)	<i>Leucaena leucocephala</i>	Fabaceae	Forage	+				20
(45)	<i>Samanea saman</i>	Fabaceae	Pods	+	+			50
(46)	<i>Enterolobium cyclocarpum</i> + <i>Gliricidia sepium</i>	Fabaceae	Pods + forage	+	+			6.3
(47)	<i>Fagopyrum esculentum</i>	Polygonaceae	Rutin				+	0
(48)	<i>Leucaena leucocephala</i>	Fabaceae	Forage	+				14
(49)	<i>Terminalia chebula</i>	Combretaceae	Seed pulp	+				13
(49)	<i>Allium sativum</i>	Amaryllidaceae	Bulb	+				2.5
(50)	<i>Mimosa caesalpiniaefolia</i>	Fabaceae	Forage	+				31.2
(50)	<i>Eucalyptus spp.</i>	Myrtaceae	Oil			+		30

### Role of Secondary Plant Metabolites on Enteric Methane Mitigation in Ruminants

Juan Carlos Ku-Vera<sup>1\*</sup>, Rafael Jiménez-Ocampo<sup>1,2\*</sup>, Sara Stephanie Valencia-Salazar<sup>3</sup>, María Denisse Montoya-Flores<sup>4</sup>, Isabel Cristina Molina-Botero<sup>5</sup>, Jacobo Arango<sup>6</sup>, Carlos Alfredo Gómez-Bravo<sup>7</sup>, Carlos Fernando Aguilar-Pérez<sup>8</sup> and Francisco Javier Solorio-Sánchez<sup>1</sup>

<sup>1</sup>Laboratory of Climate Change and Livestock Production, Department of Animal Nutrition, Faculty of Veterinary Medicine and Animal Science, University of Yucatán, Mérida, Mexico, <sup>2</sup>National Institute for Forestry, Agriculture and Livestock Research-INIFAP, Experimental Field Valle del Guadiana, Durango, Mexico, <sup>3</sup>College of the Southern Border (ECOSUR), Livestock and Environment, San Cristóbal de las Casas, Mexico, <sup>4</sup>National Center for Disciplinary Research in Physiology and Animal Breeding, National Institute for Forestry, Agriculture and Livestock Research-INIFAP, Ajuclitán, Quetzaco, Mexico, <sup>5</sup>Department of Nutrition, Faculty of Animal Sciences, National Agrarian University La Molina, Lima, Peru, <sup>6</sup>International Center for Tropical Agriculture (CIAT), Cali, Colombia

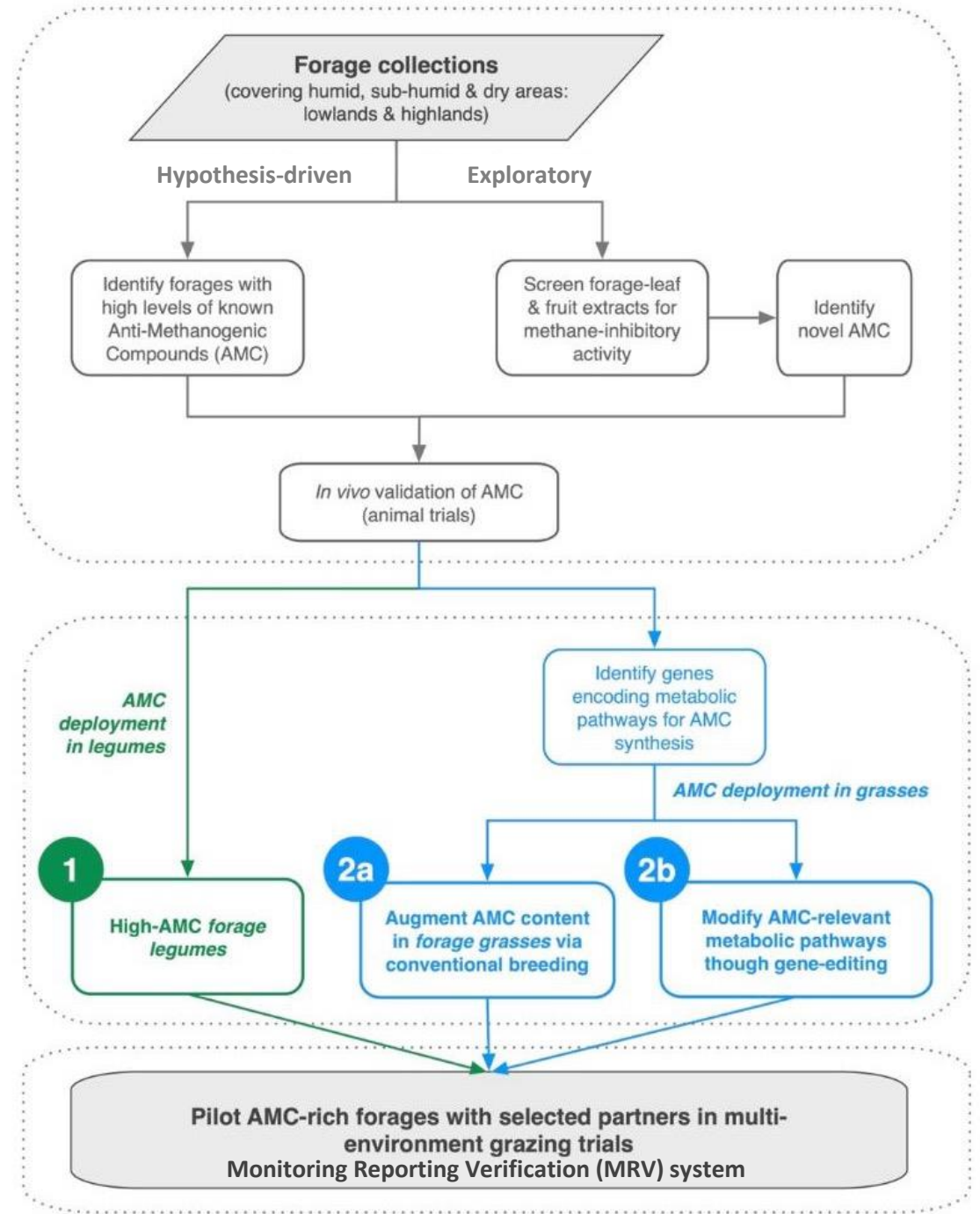
Frontiers | Role of Secondary Plant Metabolites on Enteric Methane Mitigation in Ruminants (frontiersin.org)

AMC-induced reduction of enteric CH<sub>4</sub> production by 2.5% to 50%

# Project strategy

*Main emphasis during initial 5-year project phase on WP1 (Discovery)*

Discovery (WP1)  
Development (WP2)  
Deployment (WP3)





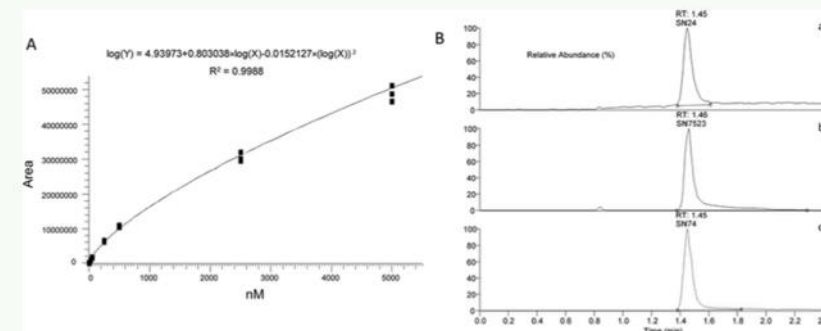
# Metabolomics Workflow



Feedstock collection and storage in freezers



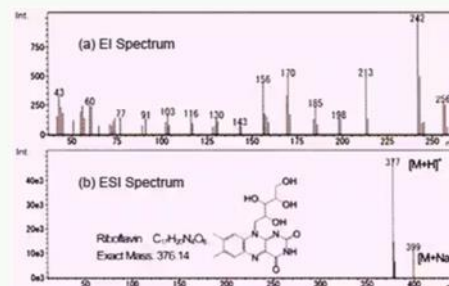
Samples processing, metabolites extraction and purification



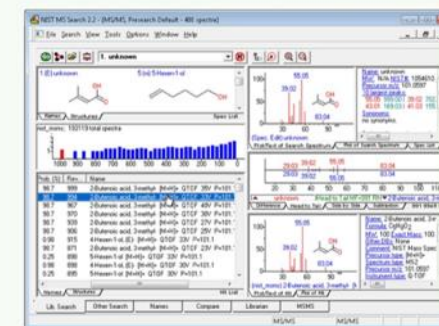
Standards calibration curves for quantification



LC MS/MS and GC FID/MS of samples



Data Treatment and Pre-processing



Data Analysis

# *In vitro* CH<sub>4</sub> production assays

## Basic setup

Incubation volume	50 ml
Incubation time	50 h
Rumen fluid	20 %
Substrate	10 mg/ml



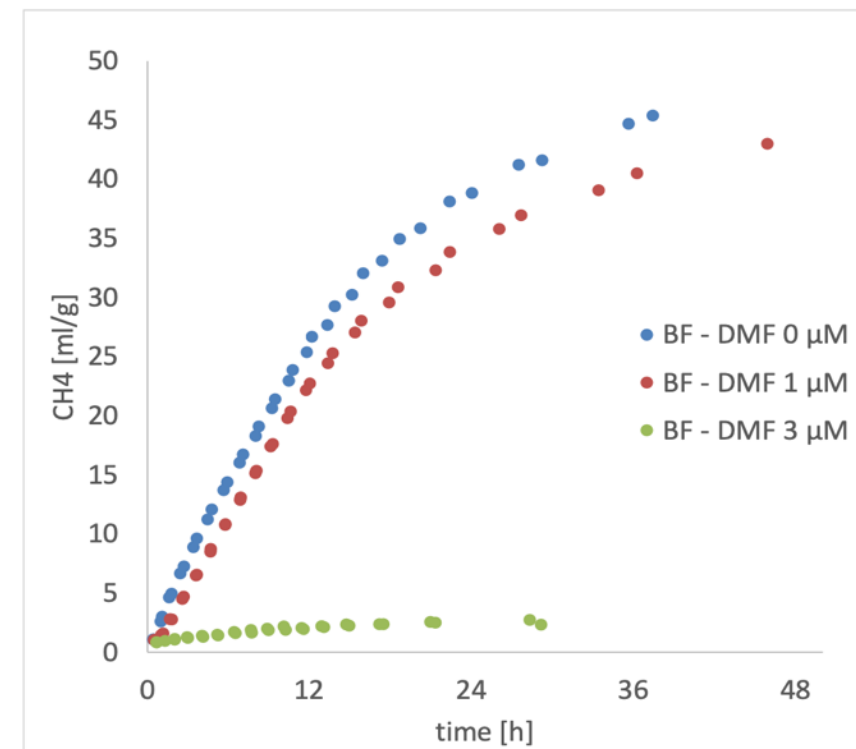
## Purpose

- Confirmation and dose response testing
- Effect of substates or solvents

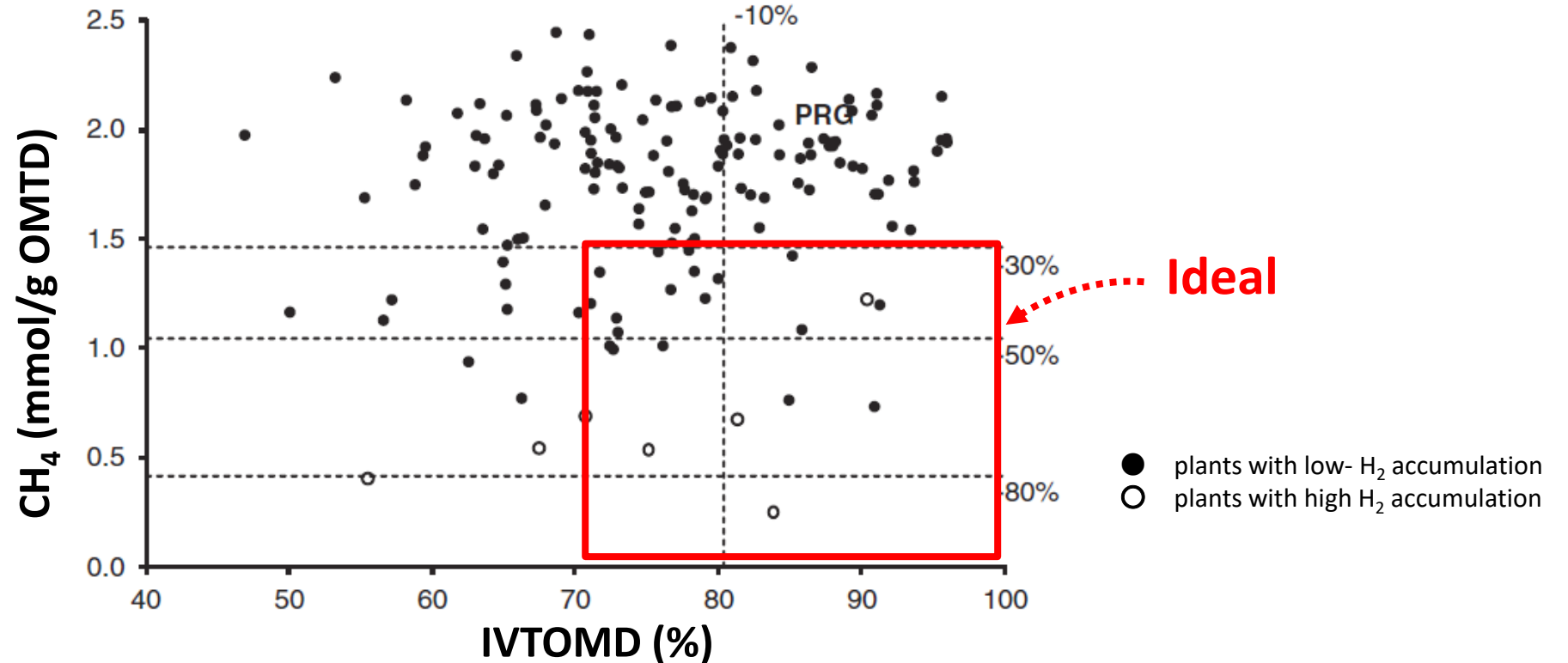
A fully automated incubation system for the measurement of gas production and gas composition

Stefan Muetzel\*, Chris Hunt, Michael H. Tavendale

AgResearch Limited, Animal Nutrition & Health, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand



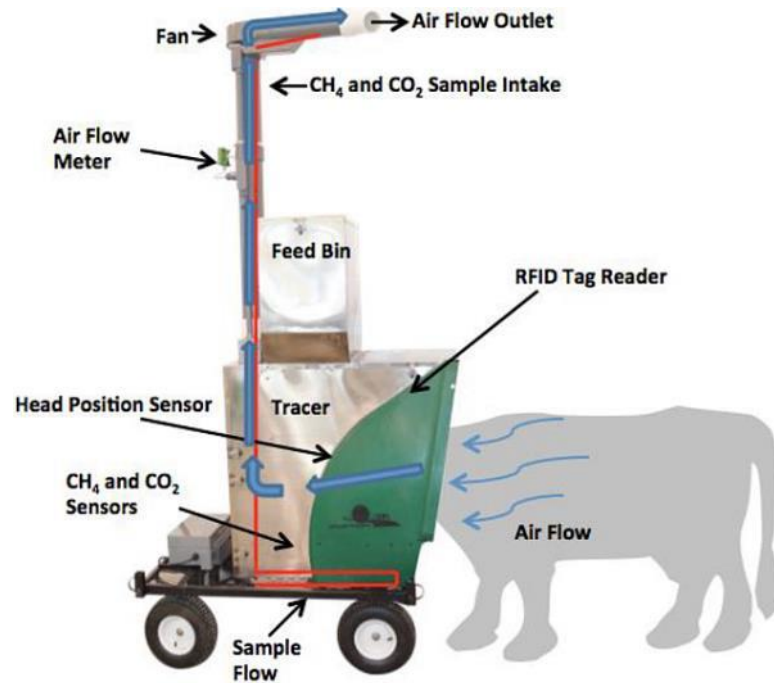
# Also need to consider digestibility



Methane production (mmol/g organic matter truly digested) and in vitro true organic matter digestibility

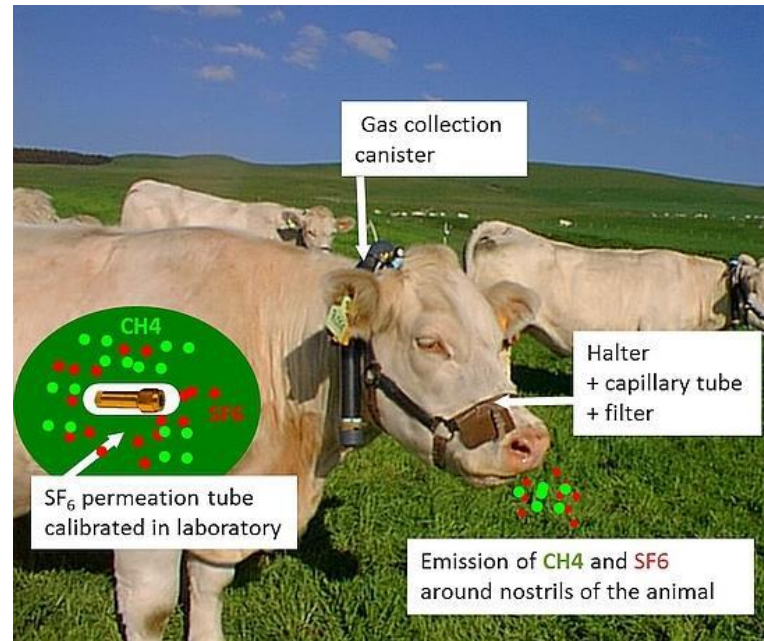
# In vivo CH<sub>4</sub> production assays

*Automated Head-Chamber System (GreenFeed)*



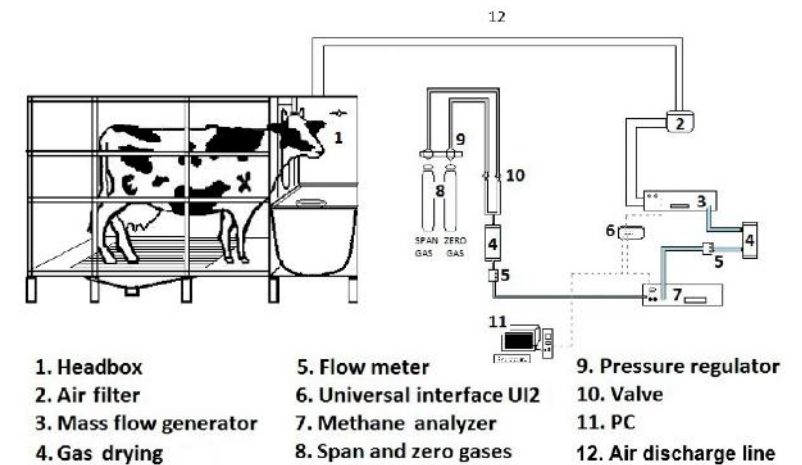
Hristov et al. (2015) . DOI: [10.3791/52904](https://doi.org/10.3791/52904)

*Sulphur hexafluoride (SF<sub>6</sub>) tracer technique*



Rochette et al. (2020) . DOI: [10.5680/mcpb013](https://doi.org/10.5680/mcpb013)

*Ventilated hood-type open circuit respiration chamber*



Castelán Ortega et al. (2020) . DOI: [10.3390/ani10020227](https://doi.org/10.3390/ani10020227)

# Envisioned project products

- AMC-rich **forage legumes** for mixed grass/legume systems
  - **Direct use/deployment** of existing varieties, accessions, or selections from accessions
- Widely used **forage grasses**
  - Increase AMC content through **gene editing** and/or **conventional breeding**
    - Alliance: *Brachiaria*, *Panicum*
    - ICARDA: barley
    - ILRI: *Cenchrus*, *Chloris*
  - Initially establishing gene-editing protocol for *Brachiaria*

# Pilot experiment starting in 2023

- Each Center to select **400 samples** including:
  - Existing, best-bet varieties
  - Maximally diverse genebank accessions
- **Validation of *in vitro* assay** for AMC activity
  - AgResearch, New Zealand
  - Each Center to establish their own *in vitro* assay platform
- Evaluate **digestibility** as a co-variate to express CH<sub>4</sub> relative to fermented organic matter
- Select best accessions for **metabolomic & nutritional analyses**
- Based on lessons learned, expand to **screen up to 10% of forage collections**

\$28 million investment

Thank you!

