



Science Fund  
of the Republic of Serbia

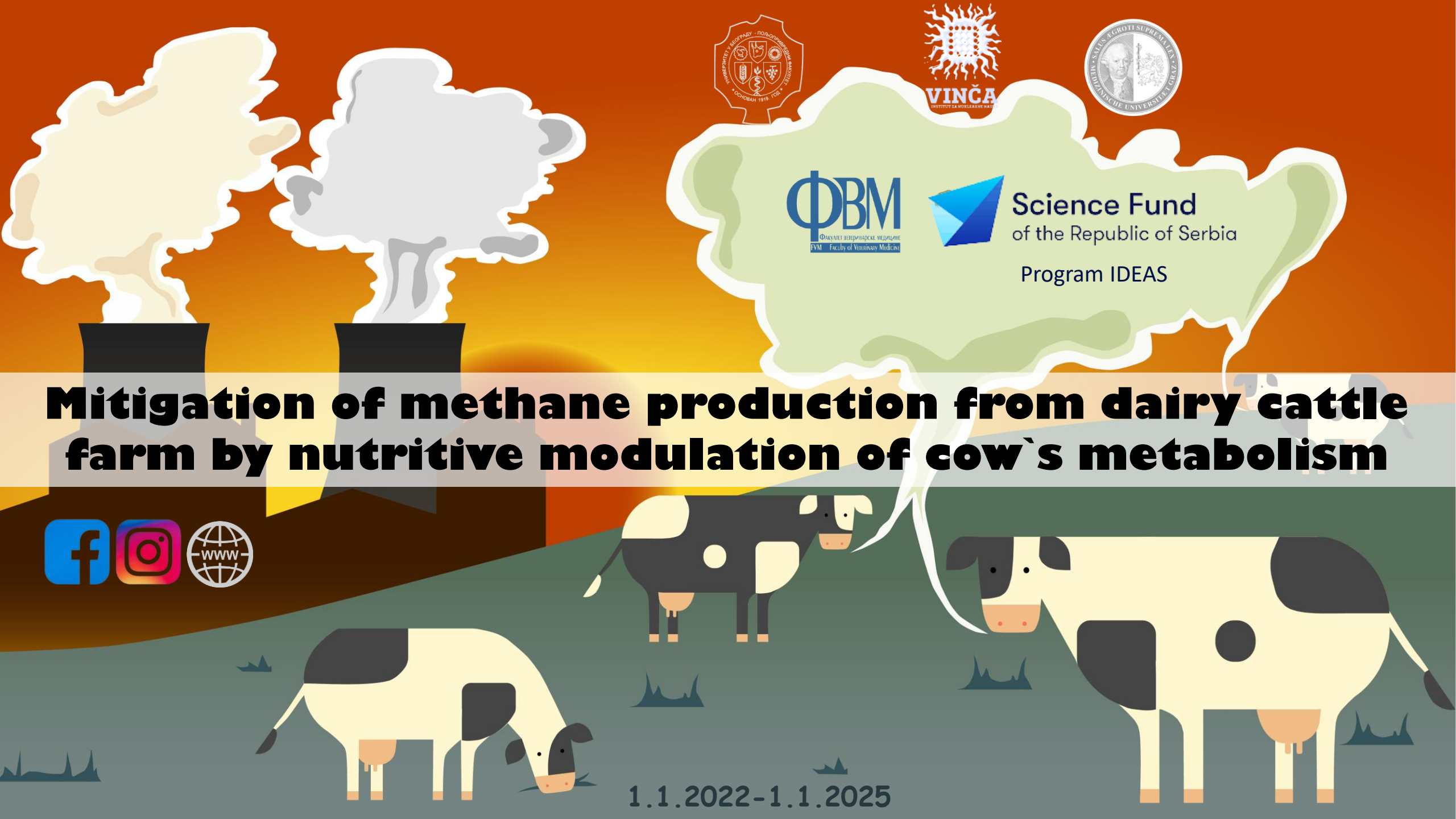
Program IDEAS

CH<sub>4</sub>

Danijela Kirovski

# **MODULATION OF COWS METABOLISM AS A TOOL FOR THE ENVIRONMENTALLY FRIENDLY CATTLE PRODUCTION**





Science Fund  
of the Republic of Serbia

Program IDEAS

# Mitigation of methane production from dairy cattle farm by nutritive modulation of cow's metabolism



1.1.2022-1.1.2025

# Our team has investigated:



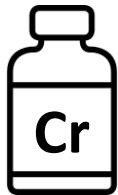
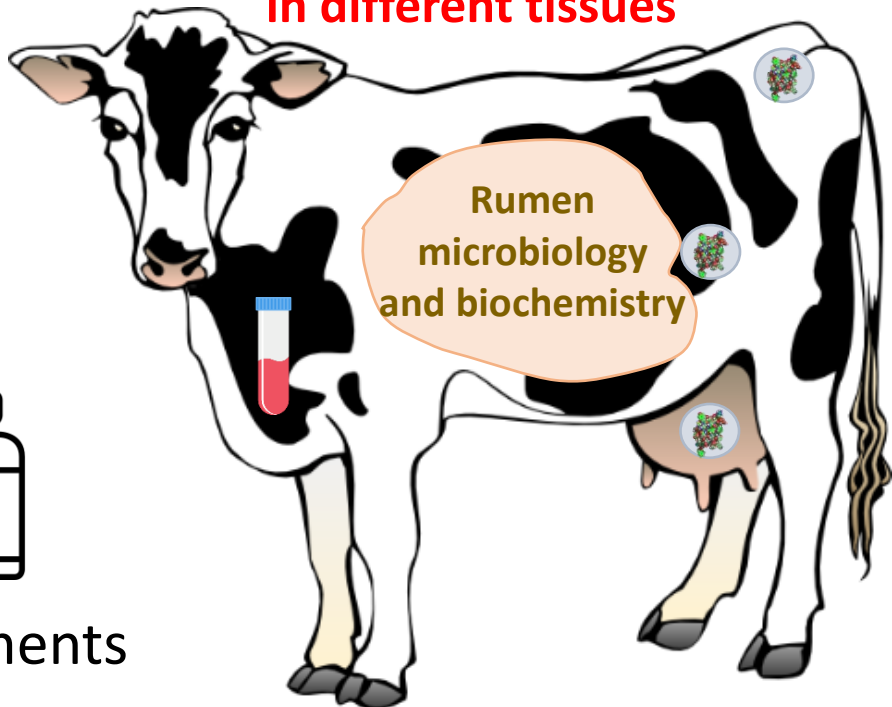
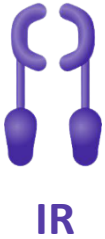
pIRS-1 Ser<sup>307</sup>  
pAkt Ser<sup>473</sup>

## Our publications

SREBP-1

Signal molecules  
in different tissues

Biopsy



Supplements



Dairy Technol. Res. (2017) 180:223–232



**J. Dairy Sci.** 93:3114–3127  
 doi:10.3168/jds.2009-2743  
 © American Dairy Science Association®, 2010.

### Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and amino acids

**A. G. Rius,\*<sup>1</sup> J. A. D. R. N. Appuhamy,\* J. Cyriac,\* D. Kirovski,† O. Becvar,‡ J. Escobar,§ M. L. McGilliard,\* B. J. Bequette,# R. M. Akers,\* and M. D. Hanigan\*<sup>2</sup>**

<sup>\*</sup>Department of Dairy Science, Virginia Polytechnic Institute and State University, Blacksburg 24061  
<sup>†</sup>Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia  
<sup>‡</sup>Department of Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg 24061  
<sup>§</sup>Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University, Blacksburg 24061  
<sup>#</sup>Department of Animal and Avian Sciences, University of Maryland, College Park 20742

#### ABSTRACT

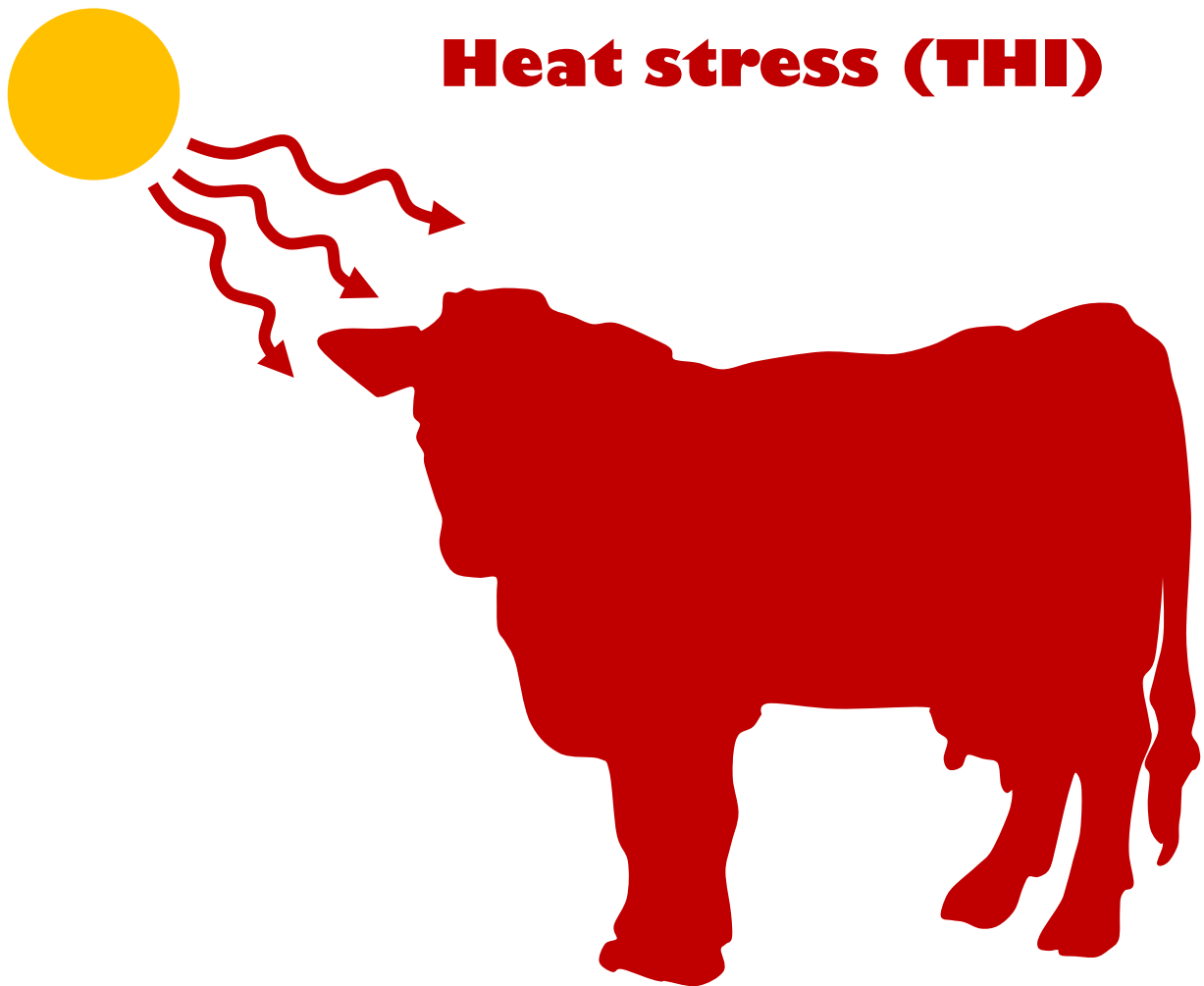
The objective of this study was to evaluate local molecular adaptations proposed to regulate protein synthesis in the mammary glands. It was hypothesized that AA and energy-yielding substrates independently regulate AA metabolism and protein synthesis in mammary glands by a combination of systemic and local mechanisms. Six primiparous mid-lactation Holstein cows with ruminal cannulas were randomly assigned to 4 treatment sequences in a replicated incomplete 4 × 4 Latin square design experiment. Treatments were abomasal infusions of casein and starch in a 2 × 2 factorial arrangement. All animals received the same basal diet (17.6% crude protein and 6.61 MJ of net energy for lactation/kg of DM) throughout the study. Cows were restricted to 70% of ad libitum intake and abomasally infused for 36 h with water, casein (0.86 kg/d), starch

Infusions of starch increased plasma concentrations of glucose, insulin, and insulin-like growth factor-I. Starch infusions increased phosphorylation of ribosomal protein S6 and endothelial nitric oxide synthase, consistent with changes in milk protein yields and plasma flow, respectively. Phosphorylation of the mammalian target of rapamycin was increased in response to starch only when casein was also infused. Thus, cell signaling molecules involved in the regulation of protein synthesis differentially responded to these nutritional stimuli. The hypothesized independent effects of casein and starch on animal metabolism and cell signaling were not observed, presumably because of the lack of a milk protein response to infused casein.

**Key words:** amino acid, cell signaling, mammary gland

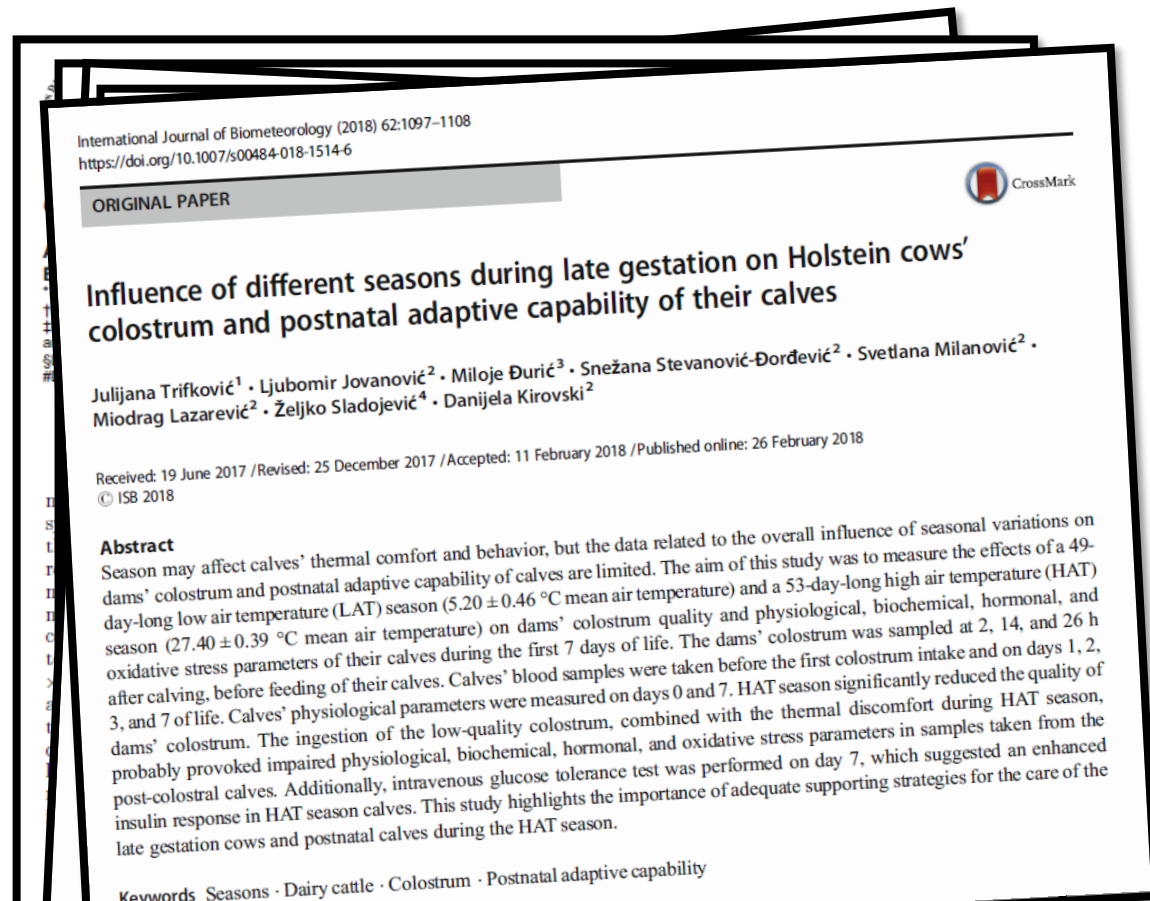
INTRODUCTION

# Our team has investigated:



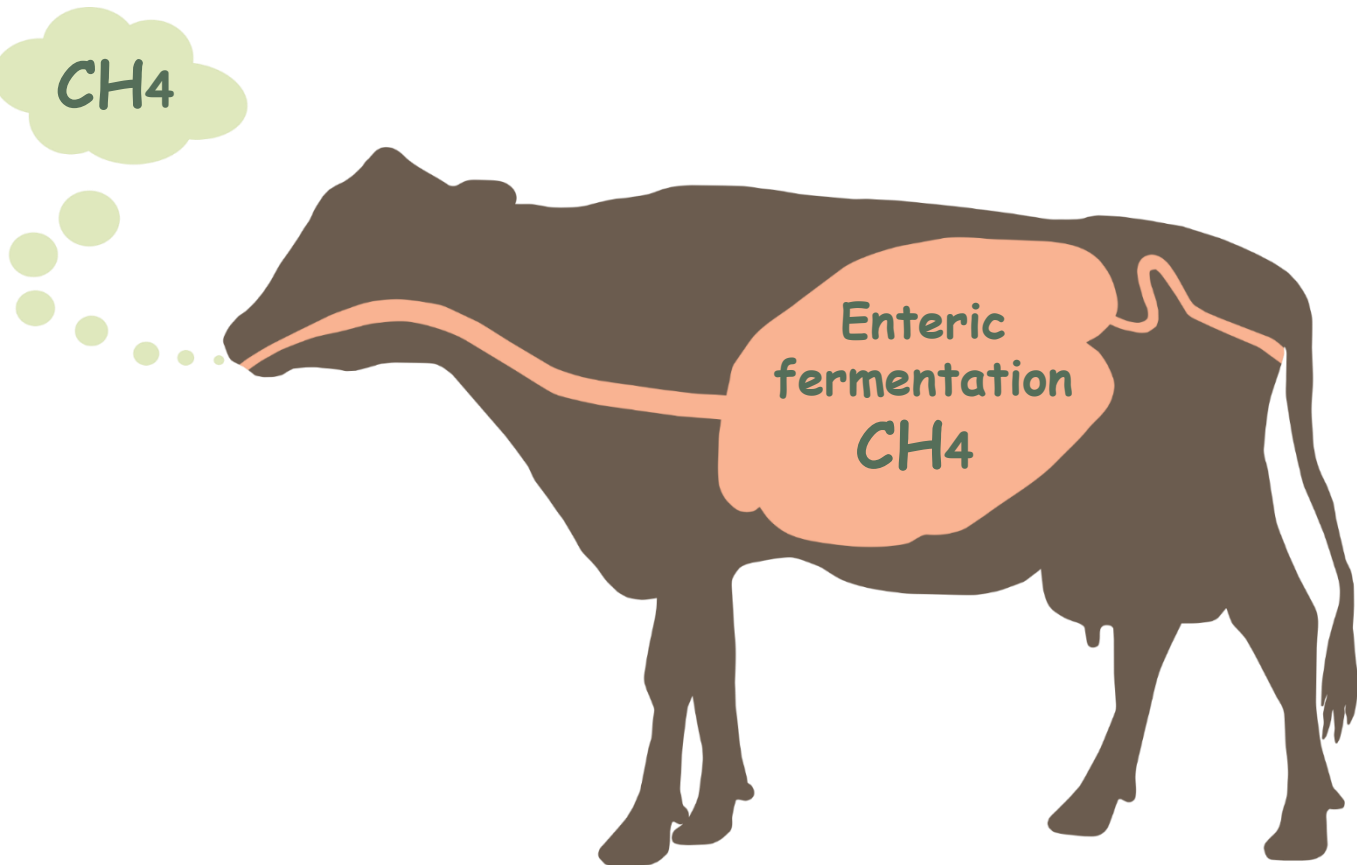
**Heat stress (THI)**

## Our publications



# Our team has investigated:

## Enteric methane emissions



## Our publications

An illustration of a document page with a black border. At the top, there are two stylized trees with blue foliage and brown trunks. The right tree has a brown chimney on its trunk, emitting a plume of blue smoke. The document page contains the following text:

Acta Veterinaria-Beograd 2023, 73 (1), 71-86  
UDK: 636.2.09:616-008.9:547.211  
DOI: 10.2478/acve-2023-0006

**sciendo**  
Research article

**METHANE EMISSION AND METABOLIC STATUS IN PEAK LACTATING DAIRY COWS AND THEIR ASSESSMENT VIA METHANE CONCENTRATION PROFILE**

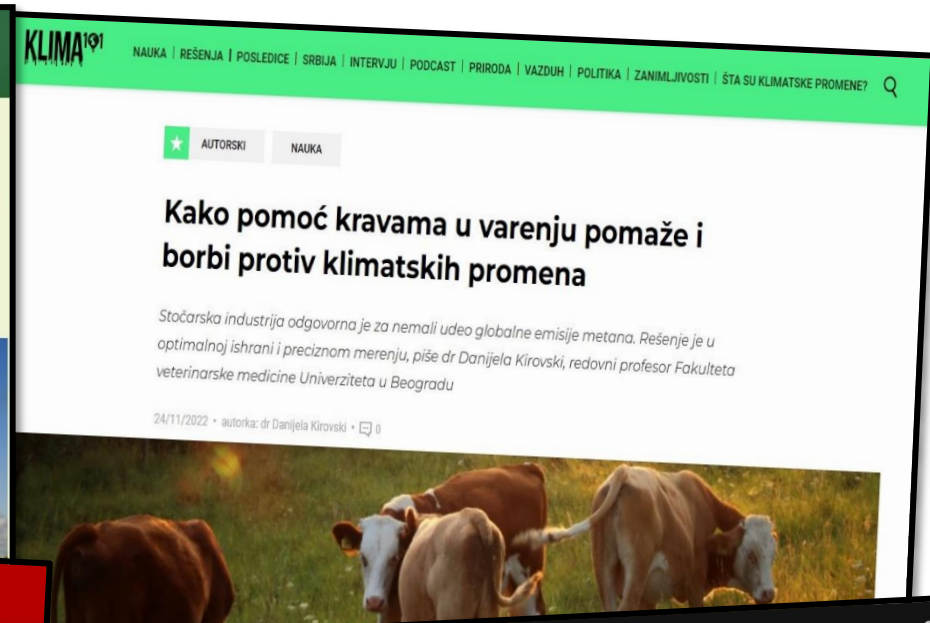
Dušan BOŠNJAKOVIĆ<sup>1</sup>, Danijela KIROVSKI<sup>1</sup>, Radiša PRODANOVIĆ<sup>2</sup>,  
Ivan VUJANAC<sup>2</sup>, Sveta ARSIĆ<sup>2</sup>, Milica STOJKOVIĆ<sup>1</sup>, Slavica DRAŽIĆ<sup>1</sup>,  
Sreten NEDIĆ<sup>2\*</sup>, Ljubomir JOVANOVIĆ<sup>1</sup>

<sup>1</sup>University of Belgrade, Faculty of Veterinary Medicine, Department of Physiology and Biochemistry, Bnl. Oslobođenja 18, Belgrade, Serbia; <sup>2</sup>University of Belgrade, Faculty of Veterinary Medicine, Department of Ruminants and Swine Diseases, Bnl. Oslobođenja 18, Belgrade, Serbia

(Received 22 October, Accepted 21 December 2022)

Ruminant husbandry contributes to global methane (CH<sub>4</sub>) emissions and beside its negative impact on the environment, enteric CH<sub>4</sub> emissions cause a loss of gross energy intake in cows. The study is aimed to estimate CH<sub>4</sub> emission and metabolic status in dairy cows via the methane concentration profile as a tool for analyzing the CH<sub>4</sub> production pattern. The study included eighteen cows whose enteric CH<sub>4</sub> emission and during three consecutive days in three periods: 2 hours before (P1),

# GUEST APPEARANCES IN THE MEDIA



# Environment

air, water and soil where people, animals and plants live



# Environmental Pollution

any substances in water, soil, or air that degrades the natural quality of the environment; offends the senses; causes a health hazard; or impairs the usefulness of natural resources.

by natural processes or human activity (ANTHROPOGENIC)

*Green house gases (GHG)*



*GLOBAL WARMING*

*CARBONE DIOXIDE*

*METHANE*

*NITROGEN OXIDE*

*HALOCARBONES*

...





# Sources of Greenhouse Gases

**Natural - NOT POLLUTANTS**



**Production**



**Elimination**

**Anthropogenic - ARE POLLUTANTS**



**Production**



**Elimination**

# Greenhouse Effect - Natural Phenomenon

essential for the life on our planet

The average temperature on the Earth is comfortable average 15°C

For the distance of the Earth from the Sun, the average temperature should be - 18°C.

**Additional warming**

„Greenhouse effect“

Natural phenomenon

Preindustrial period

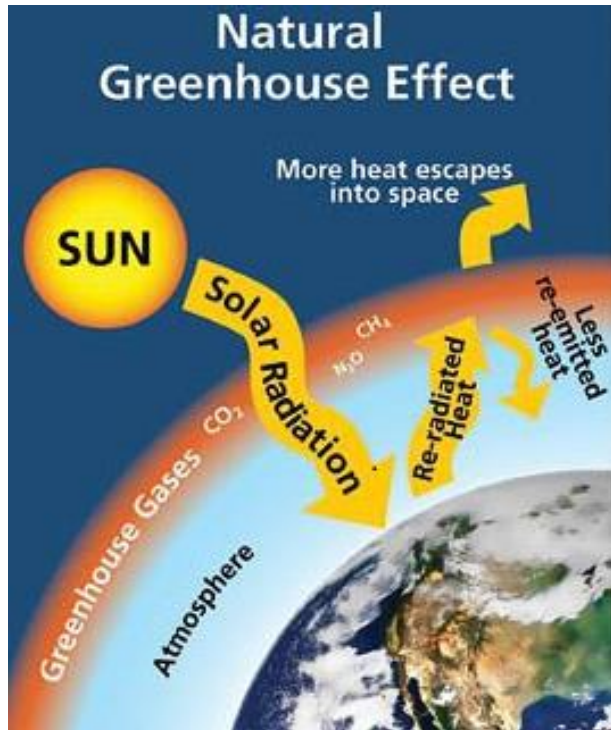
GHG conc. was 200-280 parts per mil

„Greenhouse effect“

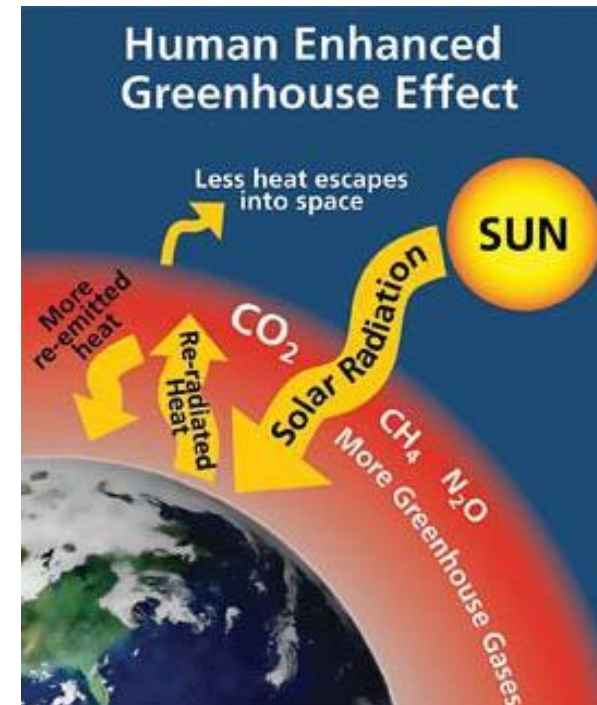
Anthropogenic reasons

Past 100 years

In 2022 GHG conc. reached more than 420 parts per million  
(50% higher than preindustrial level)

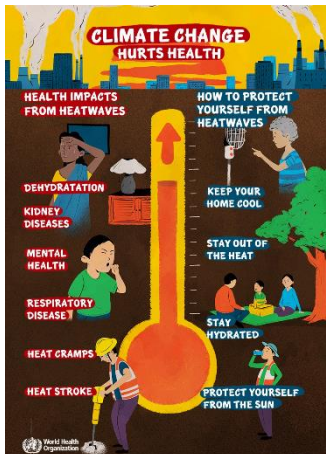
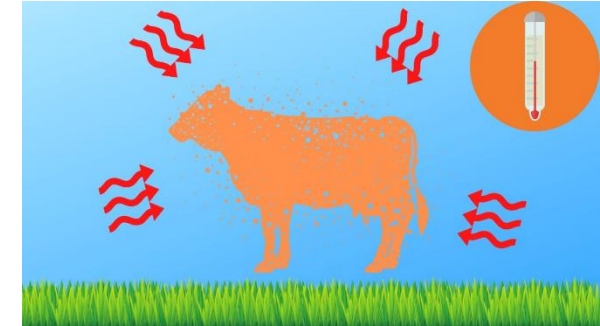
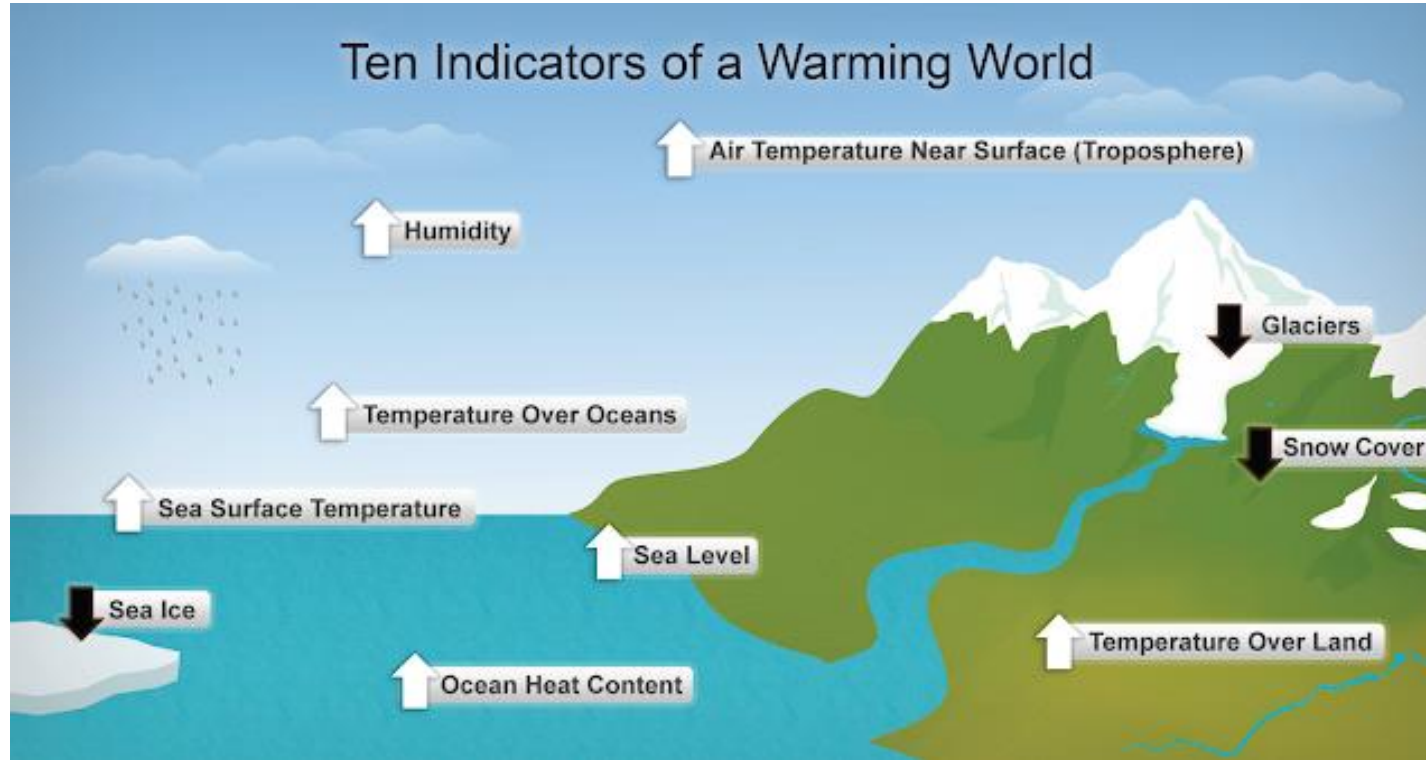
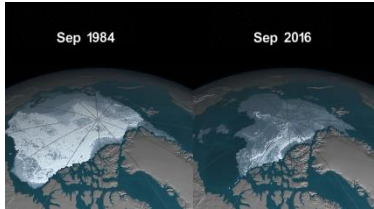


„Greenhouse“



Extra heat to be trapped  
Global temperature to rise  
Global warming to be trapped  
Global warming to be trapped  
Global warming to be trapped  
Global warming to be trapped  
**GLOBAL WARMING**

# INDICATORS OF GLOBAL WARMING



Air temperature increased by 0.8°C in the past 100 years

# CLIMATE CHANGE CONFERENCES

**Kyoto protokol (1997)** : industrialized countries and economies in transition committed that they will limit and reduce greenhouse gases (GHG) emissions in accordance with agreed individual targets (up to an average 5 per cent emission reduction compared to 1990 levels over the five year period 2008-2012).

**Doha Amendment (2012)**: agreed to take on commitments in a second commitment period (2013 to 2020)

**Paris climate conference-COP 21 (2015)**: Governments agreed a long-term goal of **keeping the increase in global temperature well below 2°C above pre-industrial levels and to aim to limit the increase to 1.5°C**

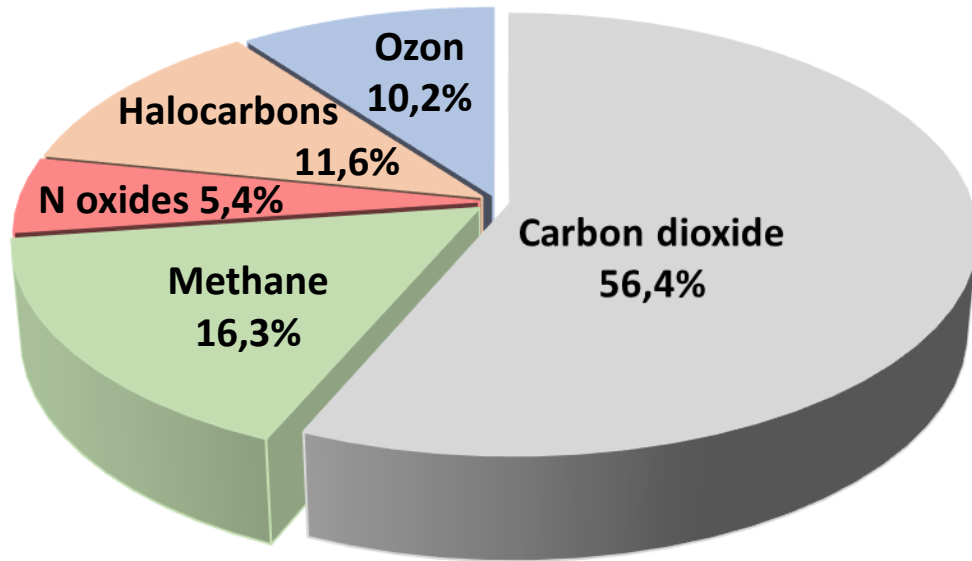
**26<sup>th</sup> UN Climate Change Conference -COP 26 (2020)**: to reach **„net zero“ of GHG emission until 2050.**



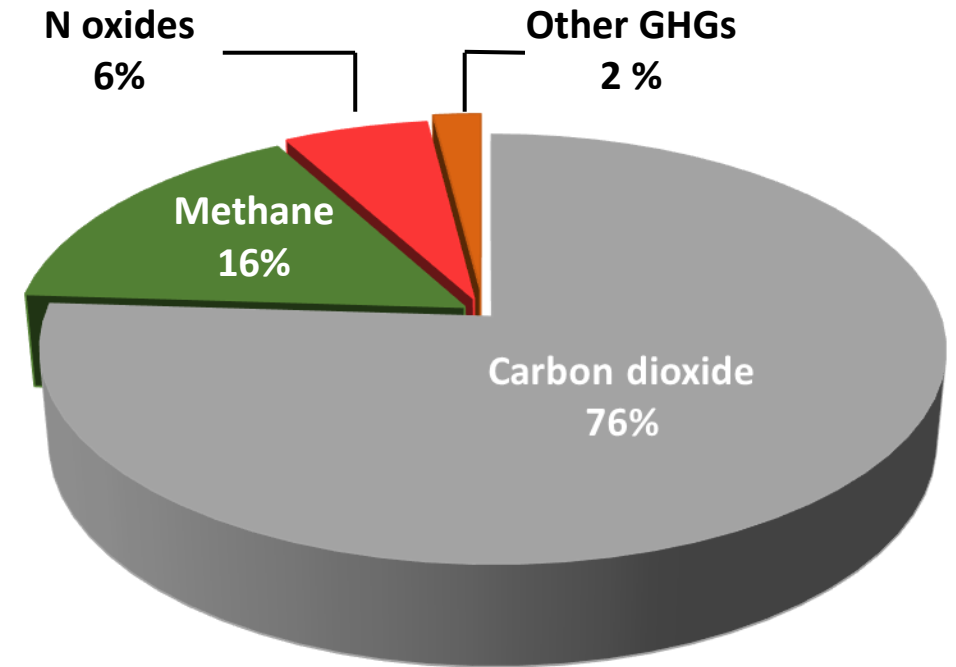
**COP 26 – limit methane emissions by 30% by 2030, compared to 2020 levels.**

# Presence of GHG in atmosphere

## Natural



## Anthropogenic - accumulation in the atmosphere



# Global warming potential - GWP

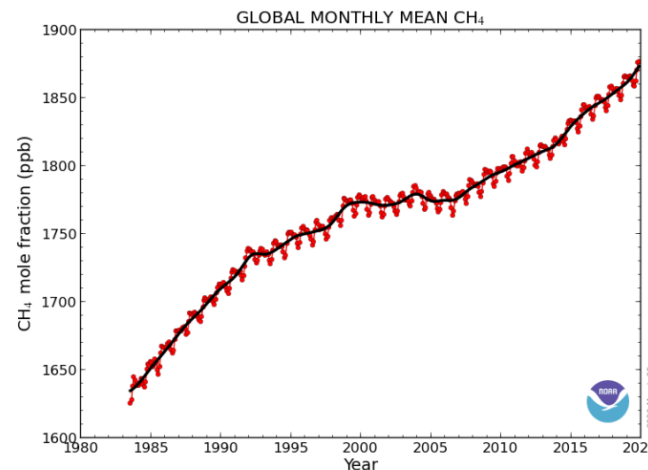
A measure of how much energy the emissions of 1 ton of a gas will absorb over a given period of time, relative to the emissions of 1 ton of carbon dioxide (CO<sub>2</sub>eq). So, a CO<sub>2</sub> eq is a unit used to standardize the climate effects of various GHG.

Gas	Half-life (years)	GLOBAL WARMING POTENTIAL		
		GWP <sub>20</sub>	GWP <sub>100</sub>	GWP <sub>500</sub>
CO <sub>2</sub>	1000	1	1	1
CH <sub>4</sub>	10-12	56	21	6,5
N <sub>2</sub> O	120-150	280	310	170

1 kg of CH<sub>4</sub>

=

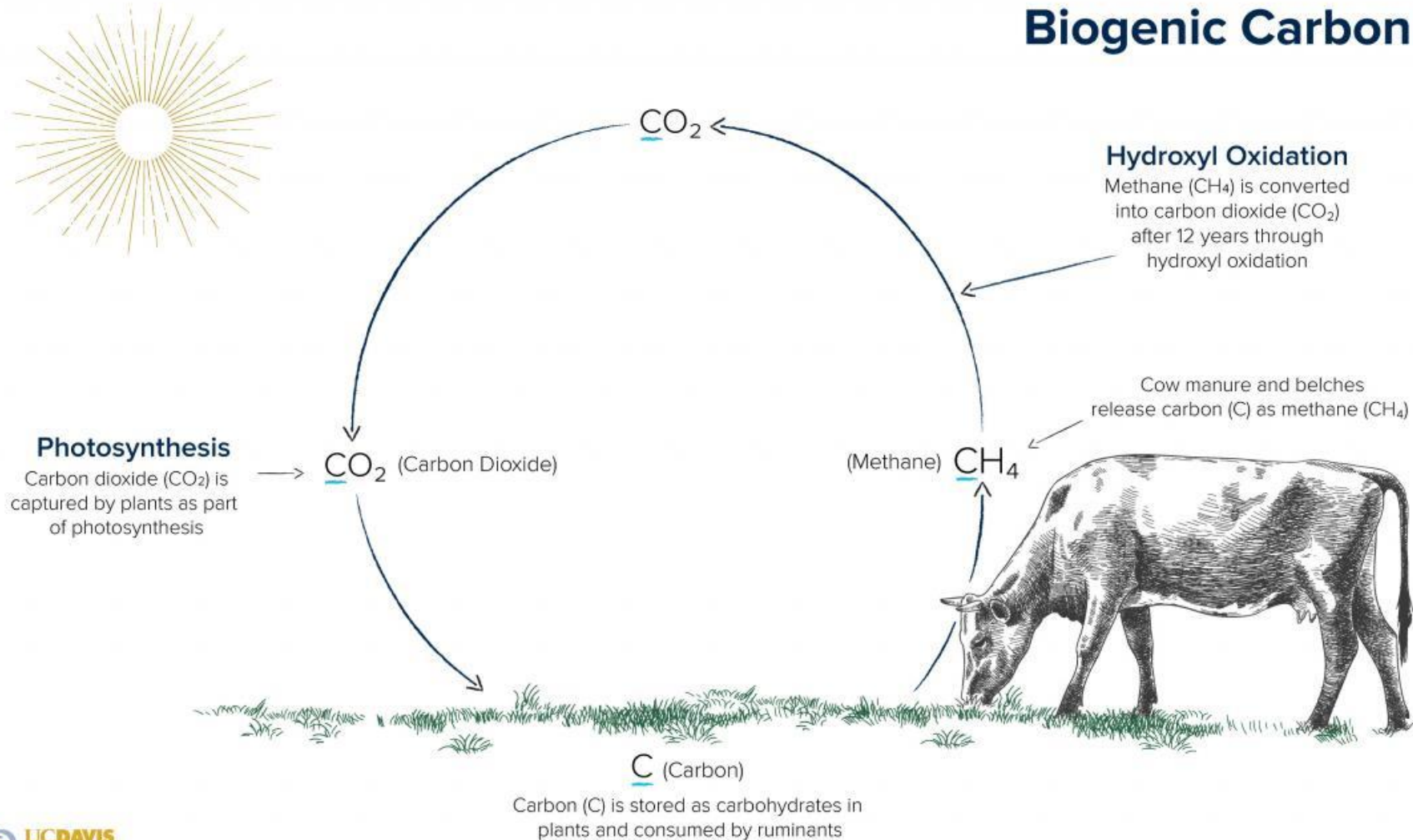
32 kg of CO<sub>2</sub>



Short-term warming  
of the earth's surface

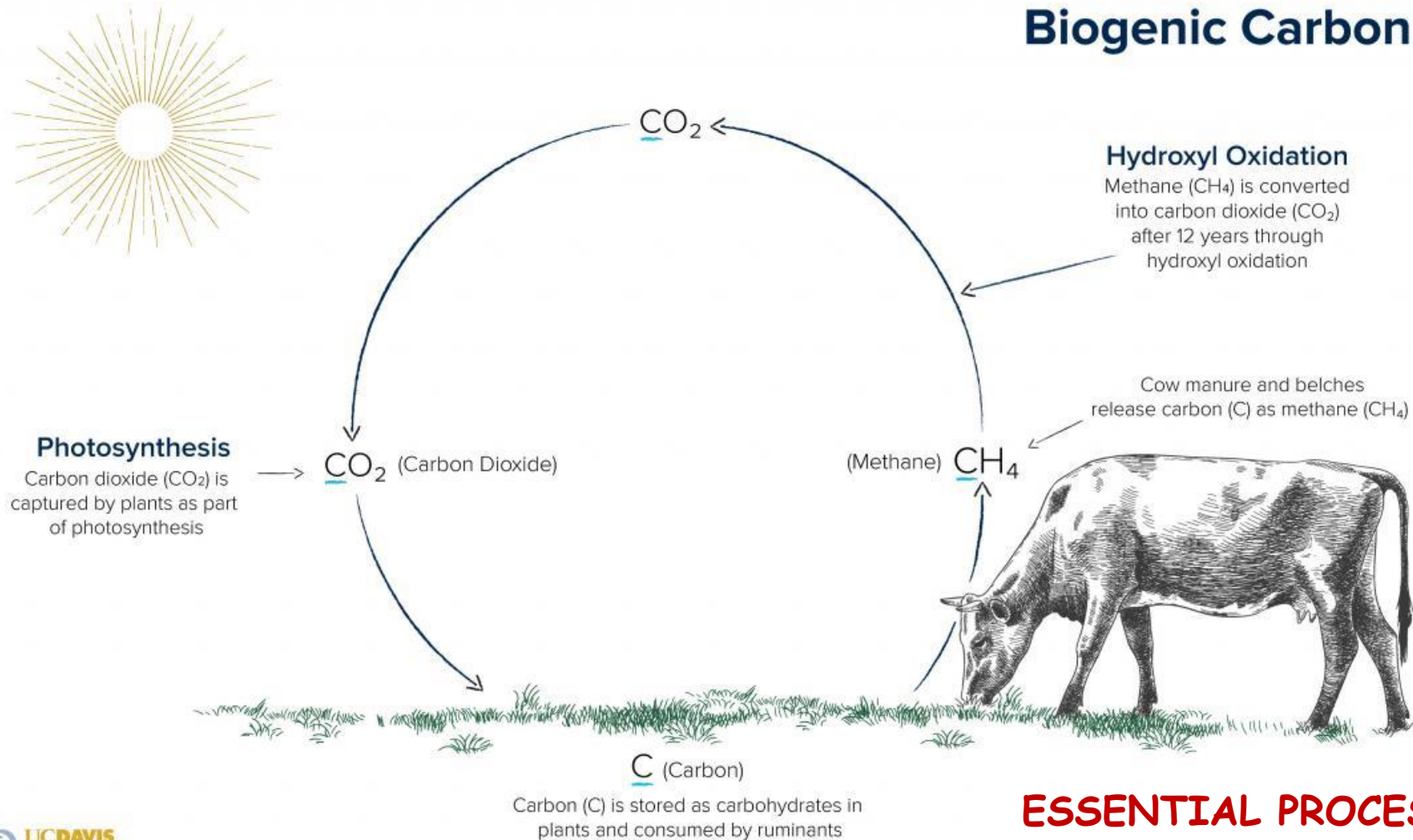
**COP 26 – reducing methane emissions by 30% by 2030.**

# Biogenic Carbon Cycle



Cellulose is the most abundant organic compound in the world (in all grasses, shrubs, crops, and trees). Its content is particularly high in grasses and shrubs found on **marginal lands, which are places where grains and other human edible crops cannot grow.**

# Biogenic Carbon Cycle



Ruminant livestock have the ability to produce high-quality human food from feedstuffs of little or no value for humans.

During enteric fermentation  $\text{CH}_4$  is produced.



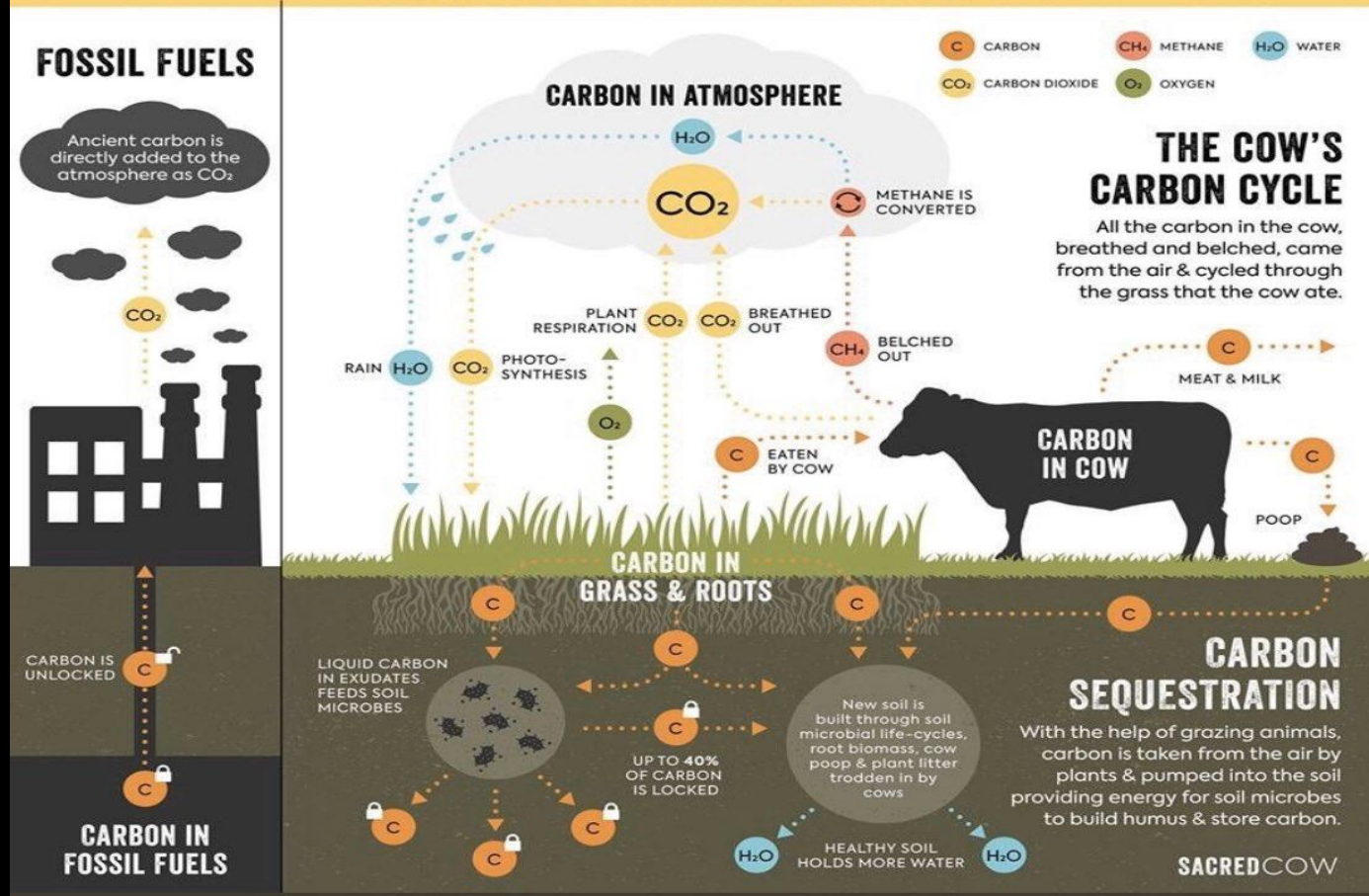
**ESSENTIAL PROCESS FOR HUMAN POPULATION!**

Two-thirds of all agricultural land is marginal, full of cellulose dense grasses that are indigestible to humans. Ruminants can digest cellulose.



# CATTLE CARBON CYCLING VS. FOSSIL FUELS

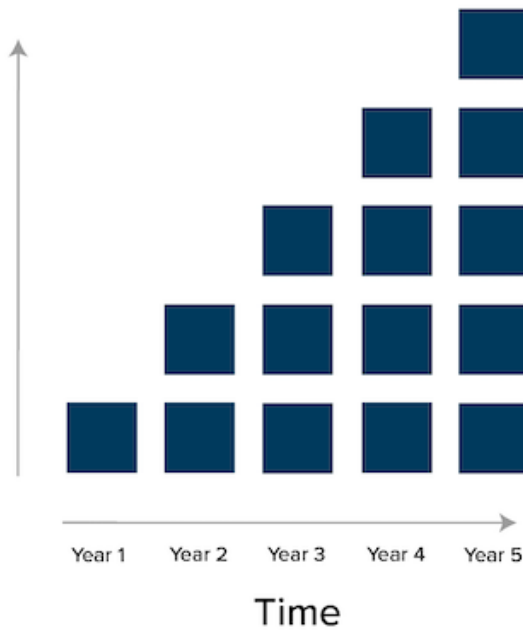
@SUSTAINABLEDISH | SACREDCOW.INFO



Biogenic carbon (which is present in methane from cows) is going through the cycle and it is very different from fossil carbon (which is present mainly in the CO<sub>2</sub> burned from fossil fuels) which goes through one way street from the bottom up into the air

■ = Pulse of CO<sub>2</sub>

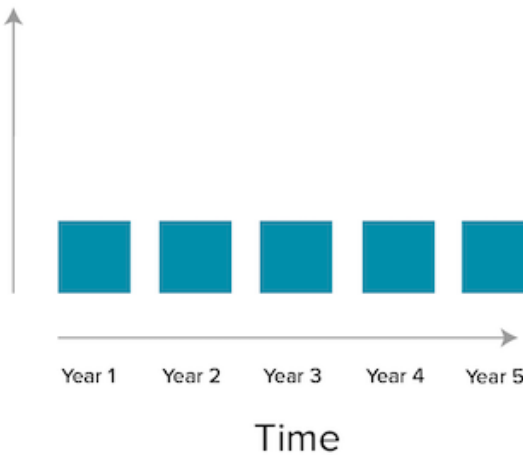
Stock  
Gas  
Carbon dioxide  
(CO<sub>2</sub>)  
Atmospheric  
Concentration



**Stock gases will accumulate over time, because they stay in the environment.**

■ = Pulse of CH<sub>4</sub>

Flow  
Gas  
Methane (CH<sub>4</sub>)  
Atmospheric  
Concentration

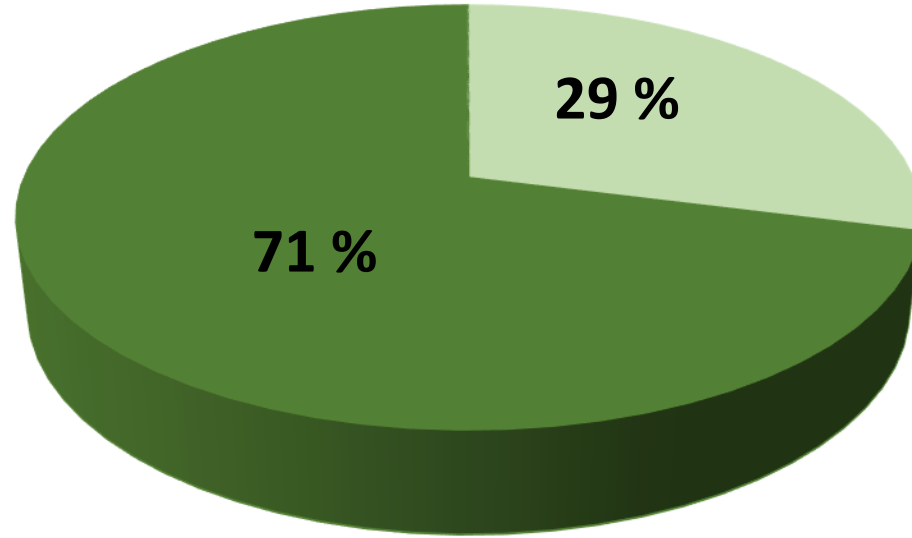


**Flow gases will stay stagnant, as they are destroyed at the same rate of emission.**

	Annual CH <sub>4</sub> emissions	Total equivalent CO <sub>2</sub> emissions	
		GWP <sub>100</sub>	GWP*
WARMING	<p>Rise by 35%</p>	<p>987 tCO<sub>2</sub>-e =33 tCO<sub>2</sub>/y for 30y</p>	<p>982 tCO<sub>2</sub>-we =33 tCO<sub>2</sub>/y for 30y</p>
STABLE	<p>Fall by 10%</p>	<p>798 tCO<sub>2</sub>-e</p>	<p>-10 tCO<sub>2</sub>-we</p>
COOLING	<p>Fall by 35%</p>	<p>693 tCO<sub>2</sub>-e</p>	<p>-562 tCO<sub>2</sub>-we</p>

If we manage to reduce methane emission from cattle such as with feed supplements we can actually **generate short term cooling** because **we pull carbon out from atmosphere and that induce cooling**

# Sources of methane

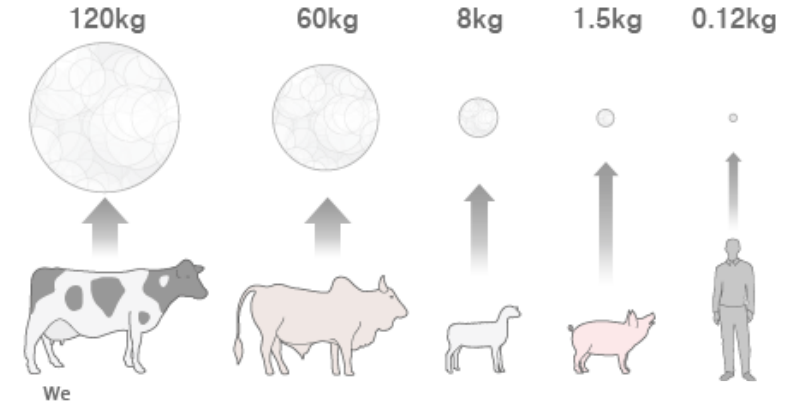


■ Natural    
 ■ Anthropogenic



Swamps (29%)

Methane emissions per animal/human per year



Oil/gas (12,7%)



Waste (12,4%)



Coal mines (6,9%)





Rice (6,9%)

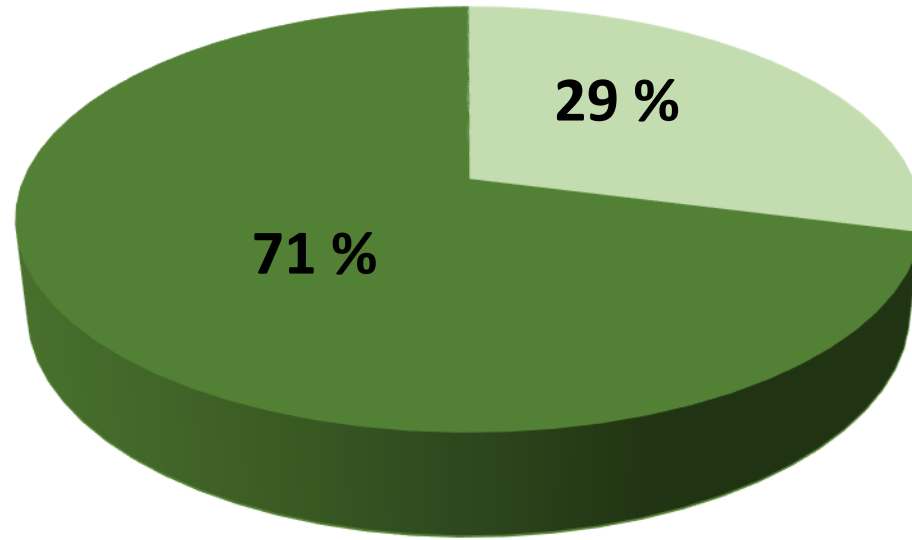


Livestock (21,3%)

**Heat Trapping Greenhouse Gases Produced by Cattle and Automobiles**

<p>Average amount of methane produced by two cows each year</p>  <p>504 lbs. methane</p>	<p>Average amount of carbon dioxide produced by one car each year</p>  <p>11,592 lbs. carbon dioxide</p>
<p>=</p>	
<p>Each year, 2 cows produce as much heat trapping greenhouse gas as 1 car driven 10,000 miles.</p> <p>NWFarmsandFood.com</p>	

# Sources of methane

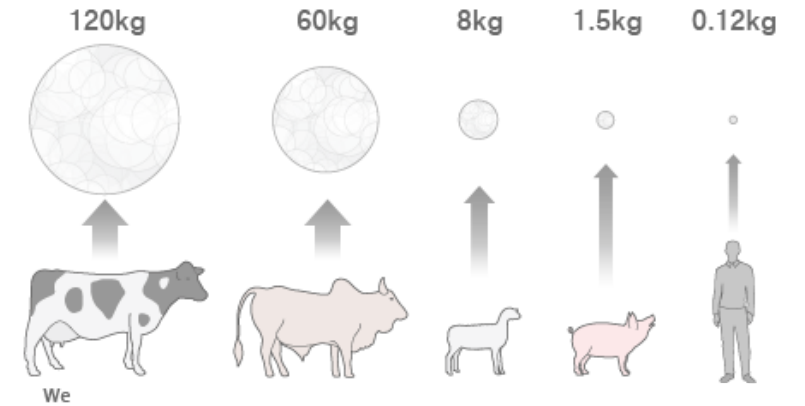


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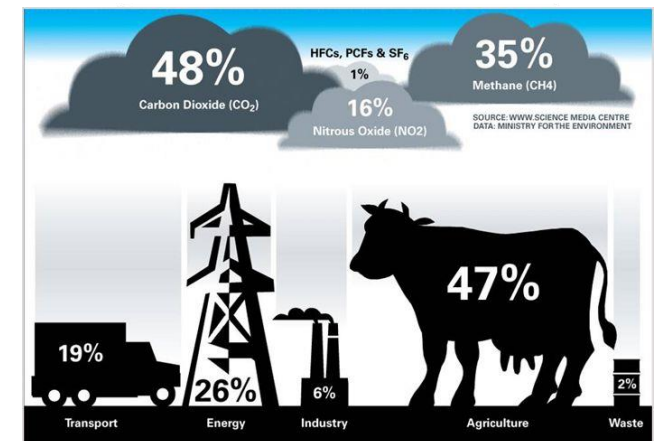
Coal mines (6,9%)



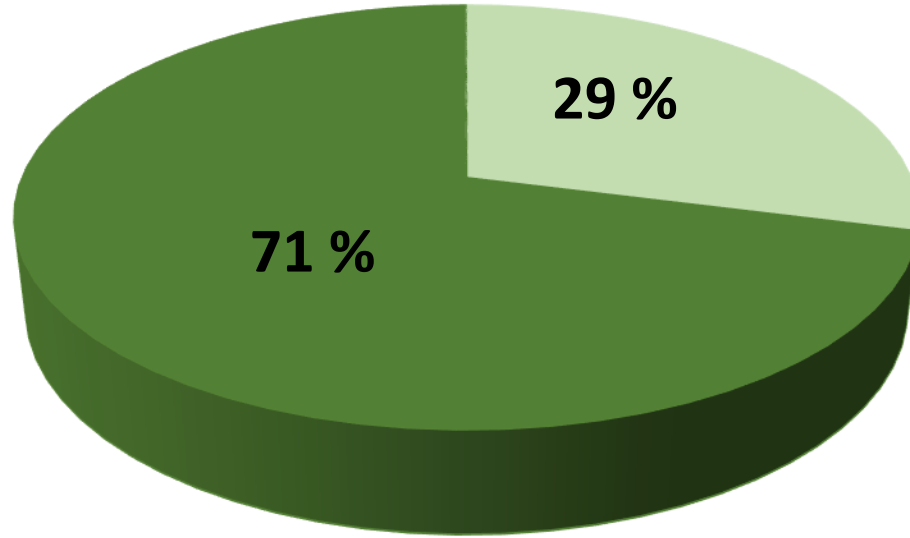
Rice (6,9%)



Livestock (21,3%)



# Sources of methane

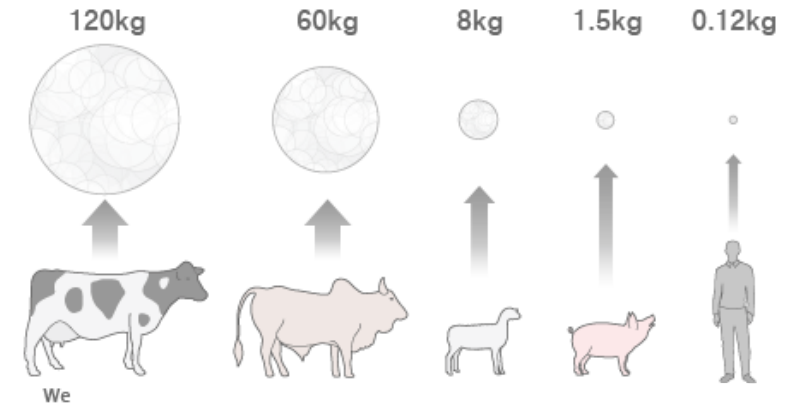


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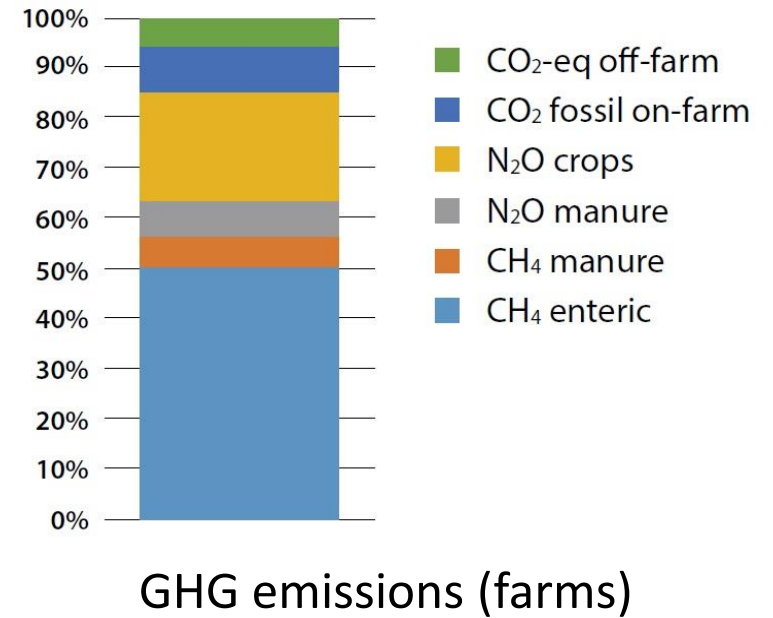
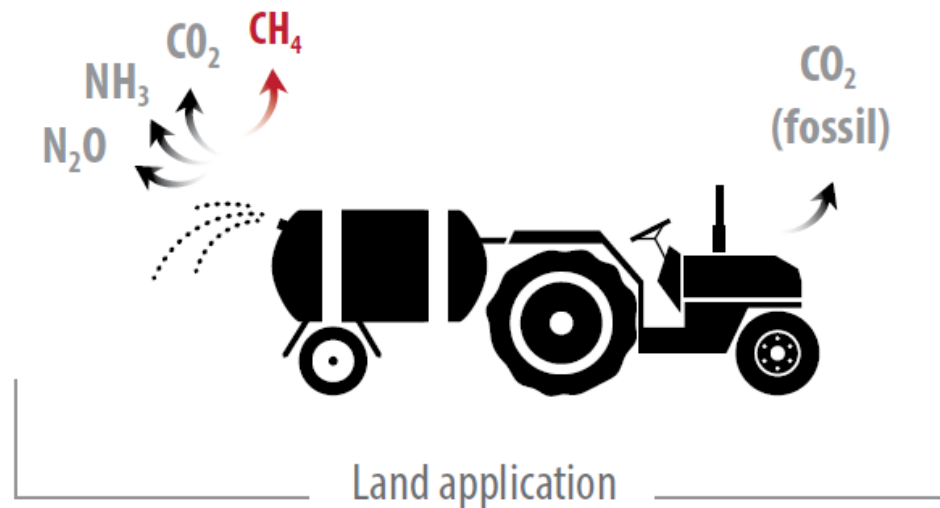
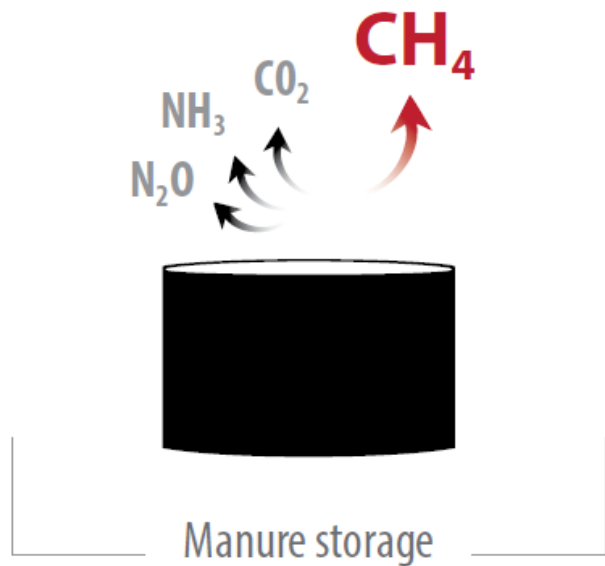
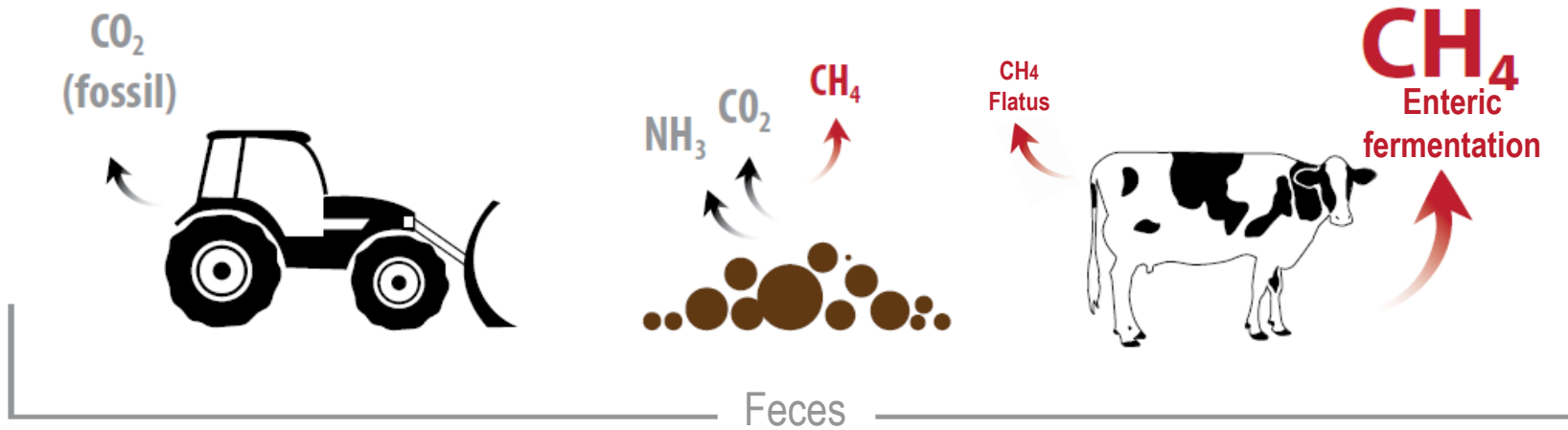
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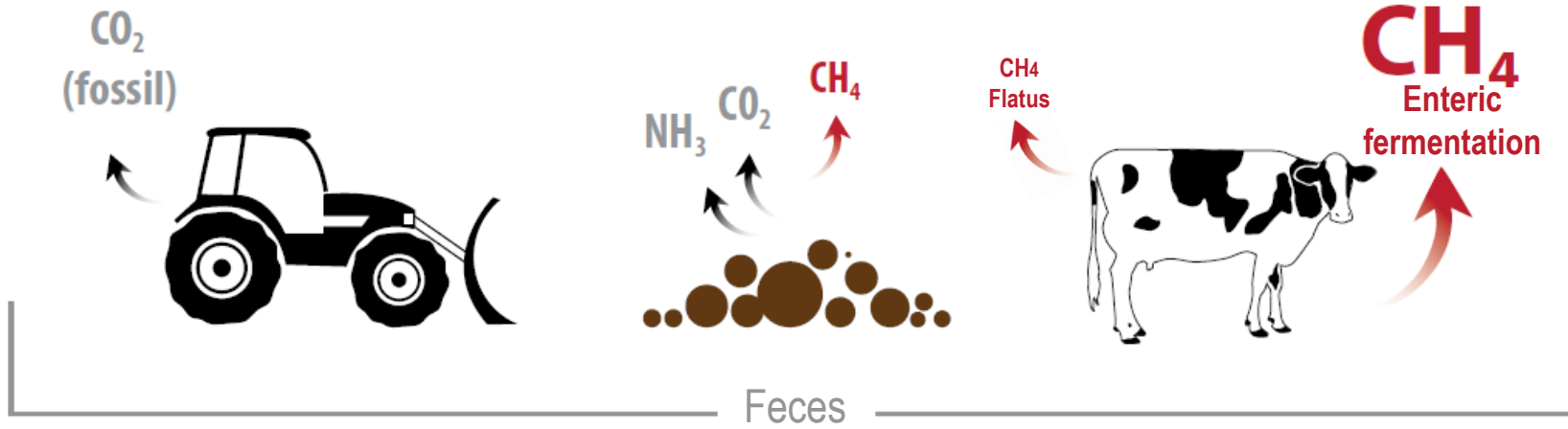
Livestock (21,3%)



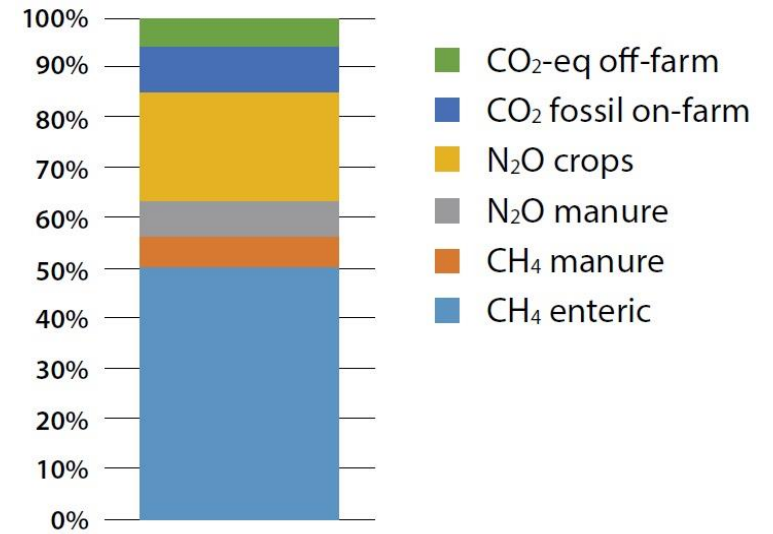
# Sources of Methane on Dairy Cow Farms



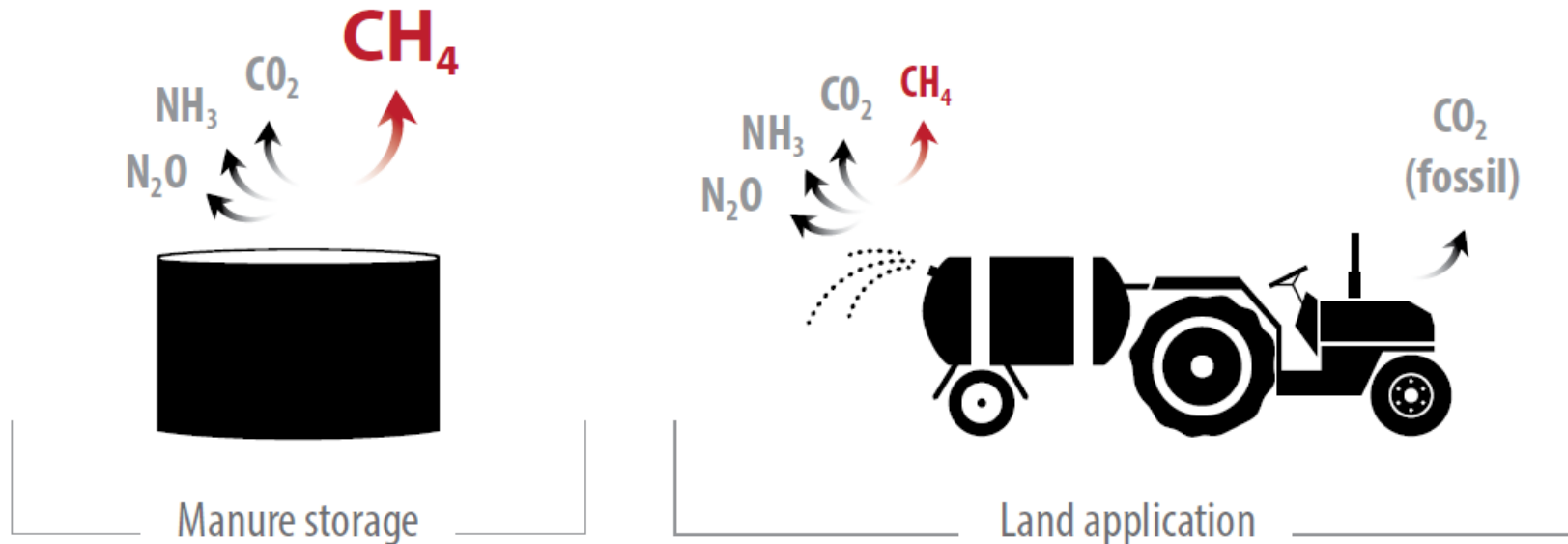
# Sources of Methane on Dairy Cow Farms



## PHYSIOLOGY



GHG emissions (farms)



## Management



# GHG Emission Measurement Methods in Ruminants

It is necessary to include the **emission factors** for individual GHGs depending on the **production category** of cattle and **part of the world**, the **size** of a given population, but also **combustion of oil (diesel)** on the farm (1 kg diesel = 3.13 kg CO<sub>2</sub>eq/kg), **electricity consumption** for milking, milk cooling, barn lighting and ventilation (0.47 kg CO<sub>2</sub>eq/kWh), **pesticide production** (22 kg CO<sub>2</sub>eq/kg pesticide), **total mass of farm machinery** (3.54 kg CO<sub>2</sub>eq/kg machine mass), etc.

$$\text{Total CH}_{4\text{Enteric}} = \sum_i E_i$$

$$\text{Emissions} = EF_{(T)} \cdot \left( \frac{N_{(T)}}{10^6} \right) \quad N_2O_{L(mm)} = \left( N_{\text{leaching-MMS}} \cdot EF_5 \right) \cdot \frac{44}{28}$$

$$N_2O_{D(mm)} = \left[ \sum_S \left[ \sum_T \left( N_{(T)} \cdot Nex_{(T)} \cdot MS_{(T,S)} \right) \cdot EF_{3(S)} \right] \right] \cdot \frac{44}{28}$$

$$CH_{4\text{Manure}} = \sum_{(T)} \frac{(EF_{(T)} \cdot N_{(T)})}{10^6}$$

$$Nex_{(T)} = N_{\text{rate}(T)} \cdot \frac{TAM}{1000} \cdot 365$$

**NUMBERS ARE APPROXIMATE NOT EXACT**

# Enteric Methane Emission Measurement Methods in Ruminants

## Inside the barn - in the facility (group production)

- The origin cannot be precisely determined
- It is not suitable for scientific research related to methane emissions
- Environmental conditions in the barn/methane emission from the facility

## Individually by animal

- Precise determination of origin
- Suitable for scientific research

# Enteric Methane Emission Measurement Methods in Ruminants

## Inside the barn - in the facility (group production)

- The origin cannot be precisely determined
- It is not suitable for scientific research related to methane emissions
- Environmental conditions in the barn/methane emission from the facility

### MULTI GAS ANALYSER



Determines up to 7 different gases ( $CH_4$ ,  $CO_2$ ,  $CO$ ,  $O_2$ ,  $H_2$ ,  $NH_3$  and  $H_2S$ ).  
The tubes draw the gas and the device measures the concentration.  
It has an internal memory for storing 500 readings.  
Results are transferred using USB, WiFi or Bluetooth.  
It has data collection software

# Enteric Methane Emission Measurement Methods in Ruminants

## Individually by animal



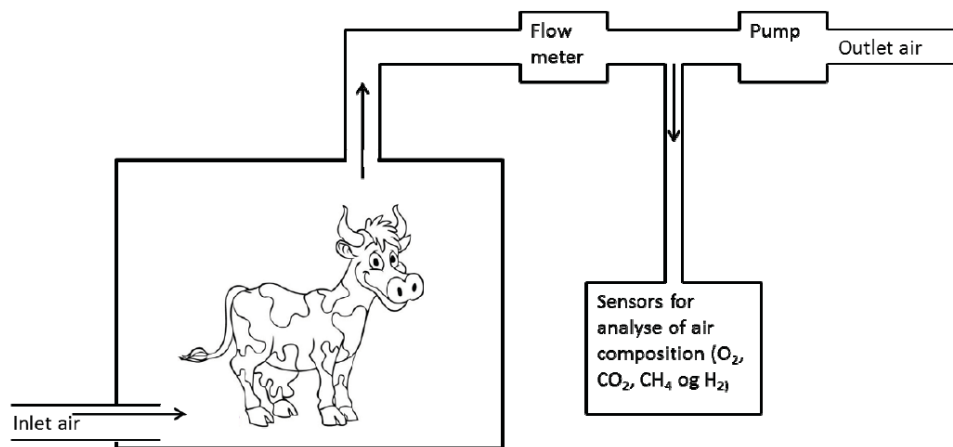
RESPIRATION CHAMBERS

### GOLD STANDARD

Initially developed to measure energy metabolism - methane emission.

Collection of exhaled air and measurement of methane concentration

Period of adaptation!



The potential issue:

**unnatural environment - question of stress and welfare**

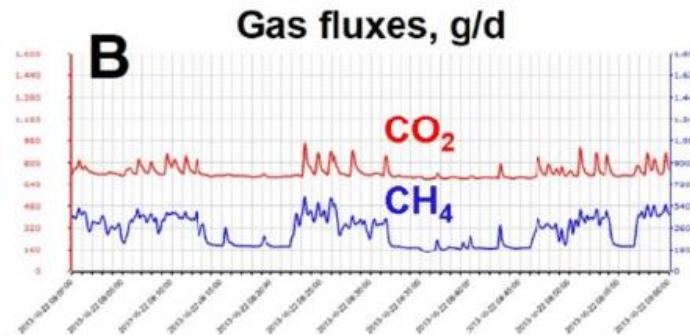
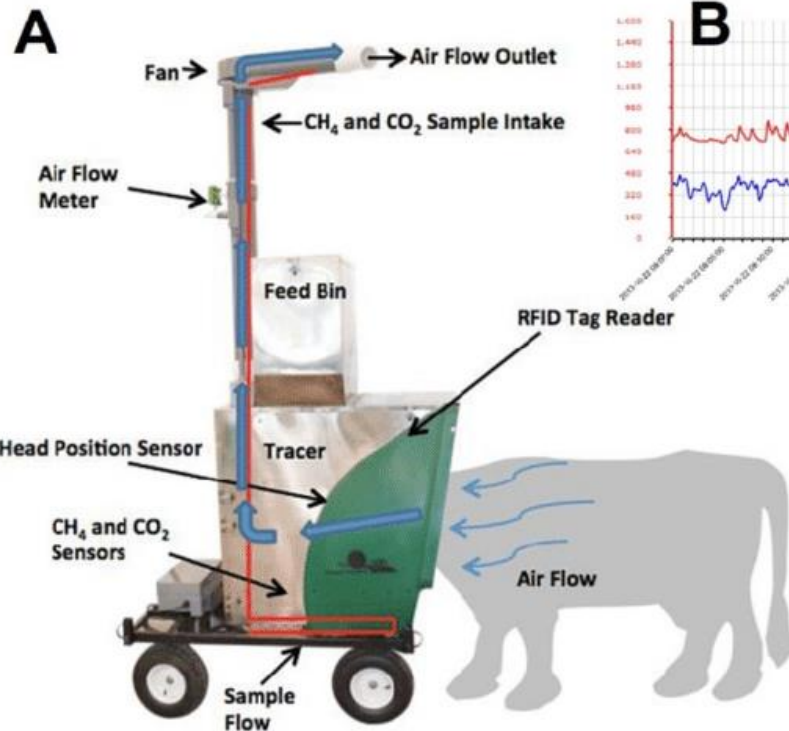
# Enteric Methane Emission Measurement Methods in Ruminants

## Individually by animal

### ALTERNATIVE METHOD

#### GreenFeed™ method

The GF system estimates daily methane production (DMP, g/day) by measuring gas concentrations and airflow over 3-7 min in exhaled air from cattle when they visit a concentrate feeders



An adaptation period is not required.  
Welfare of animal is not interrupted

# Enteric Methane Emission Measurement Methods in Ruminants

## Individually by animal

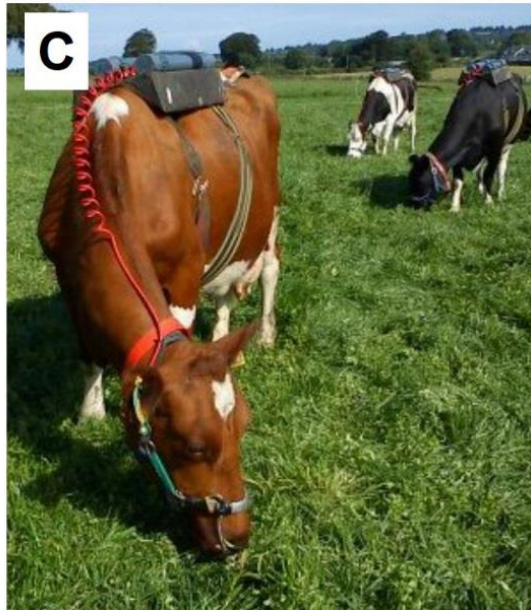
*In situ* SF<sub>6</sub> (sulfur hexafluoride) tracer method - diffusion rate

**A**

$$Q_{\text{CH}_4} = \frac{C_{\text{CH}_4} - C_{\text{CH}_4}^b}{C_{\text{SF}_6} - C_{\text{SF}_6}^b} Q_{\text{SF}_6} \frac{MW_{\text{CH}_4}}{MW_{\text{SF}_6}}$$

**B**

SF<sub>6</sub>



**FOR GRAZING ANIMALS - the device is on the animal**

A known tracer gas emission from the rumen

SF<sub>6</sub>-non-toxic and physiologically inert gas

SF<sub>6</sub>-cheap, low detection level, easy to analyze

Determination of SF<sub>6</sub> and CH<sub>4</sub> from canisters by gas chromatography

**Adaptation to the device, question of welfare**

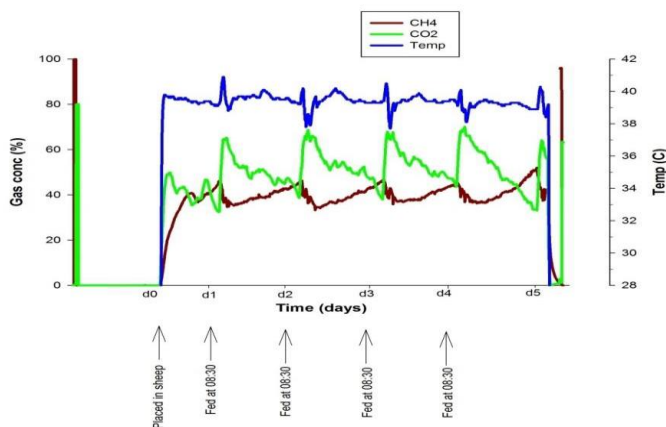
# Enteric Methane Emission Measurement Methods in Ruminants

## Individually by animal

*In situ method for measuring CH<sub>4</sub> production*



CSIRO - gas sensors for measuring CH<sub>4</sub> production in the rumen



Continuous monitoring of production in real time in the rumen  
Measures both CO<sub>2</sub> and H<sub>2</sub> and enteric gases  
They measure temperature and pH in the rumen  
It does not need to be removed from the rumen

Initially made for human population

# OUR CHOICE

Sensitivity: 1 ppm.

Measurable level: 1 - 50 000 ppm.

Measuring Speed: 0,1 s.

Measurable distance: 0,5 - 50 m.

Detection of CH<sub>4</sub> in a mixture of gases with high specificity (Chagunda, 2013).



(a)

(b)

(c)



Sorg (2021)

(d)

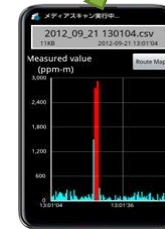
## Laser Methane Detector (infrared absorption spectroscopy)

At short intervals, a very low CH<sub>4</sub> concentration released by exhalation or a high CH<sub>4</sub> concentration eliminated by eructation may be detected.

Non-contact measurement of CH<sub>4</sub> emission (animal welfare and operator safety). Minimal interference with the comfort of the animals and their natural behavior..

Suitable for measuring in barns where other gases are present (high specificity)

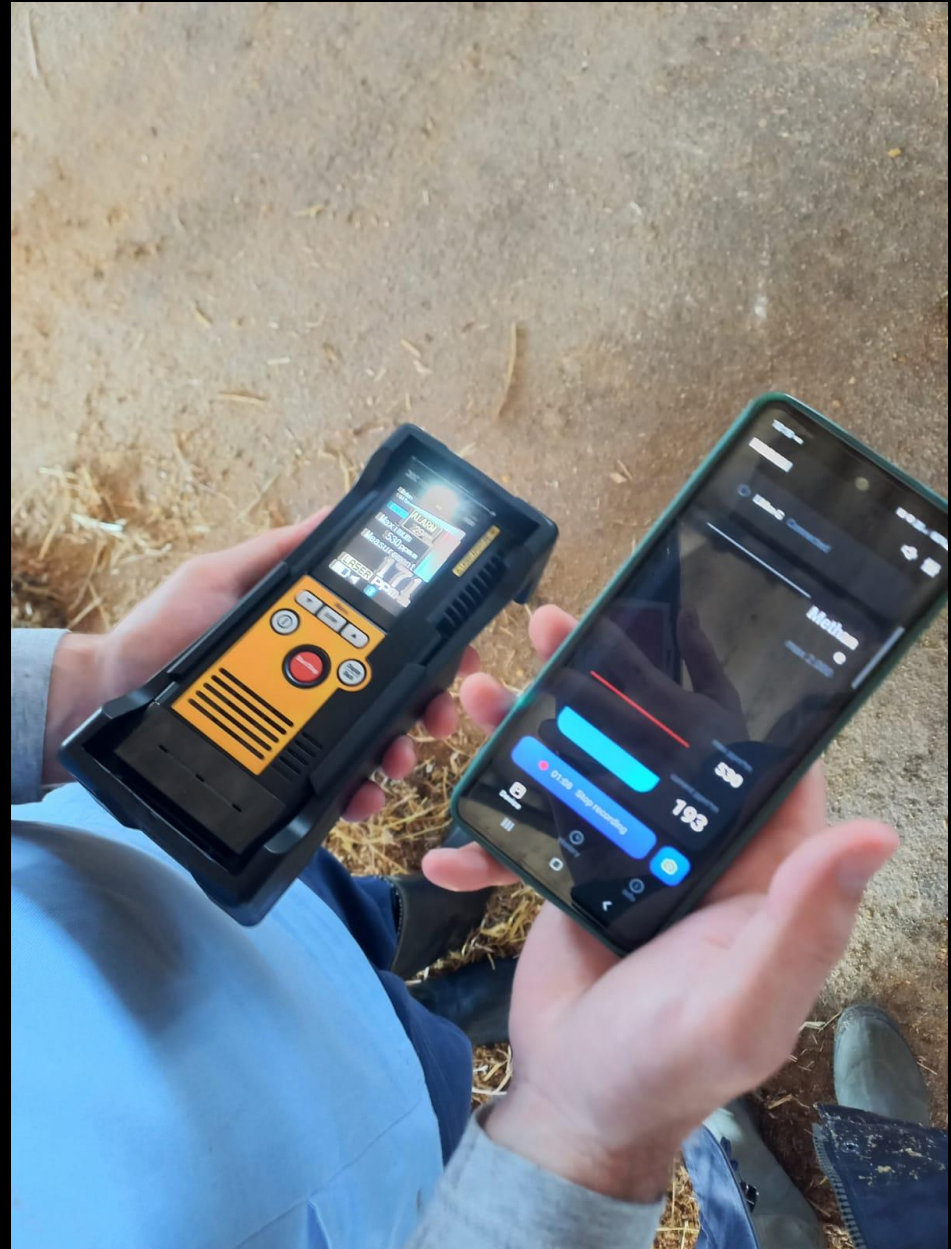
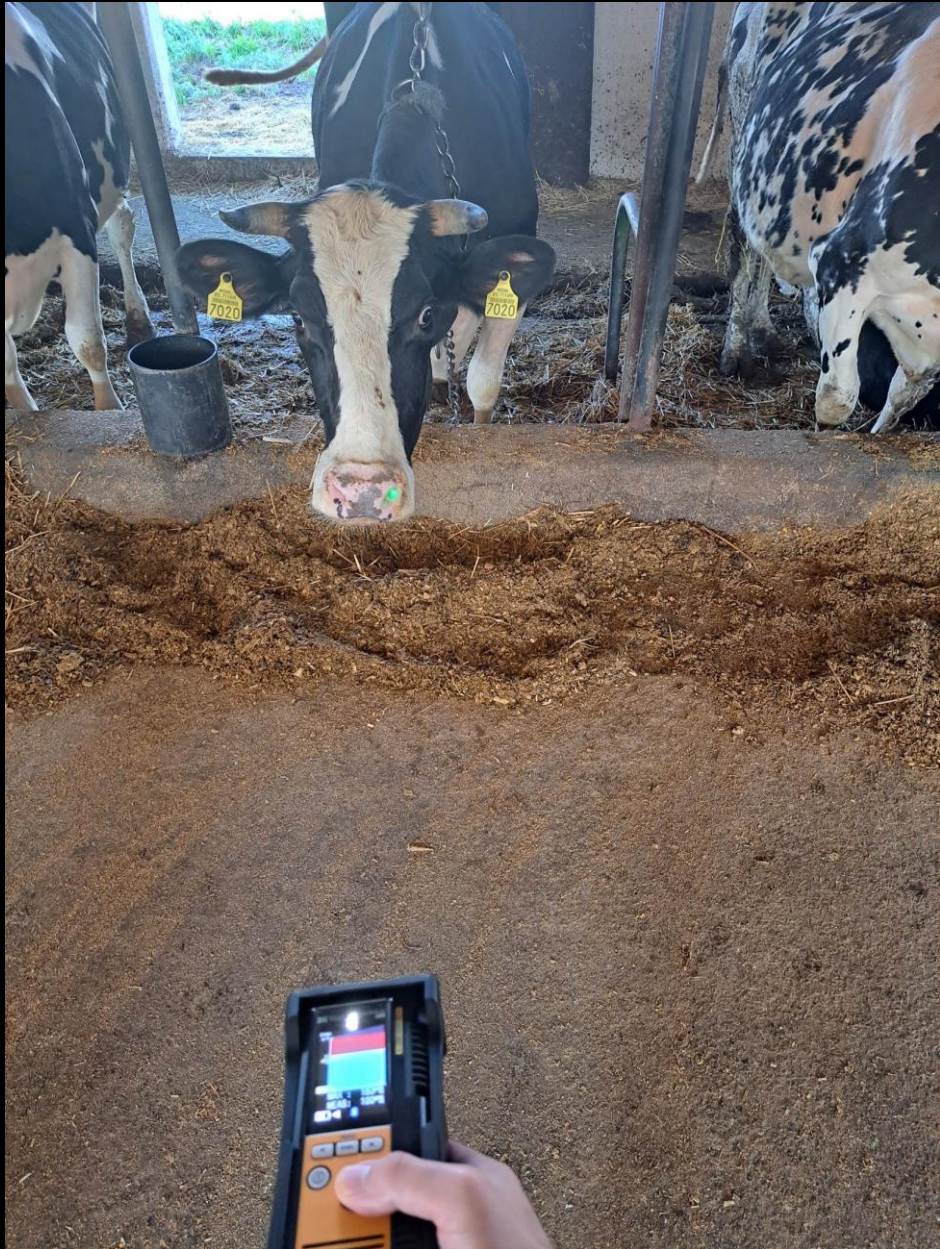
It is easy to handle, and emits laser beam (green - excellent visibility).

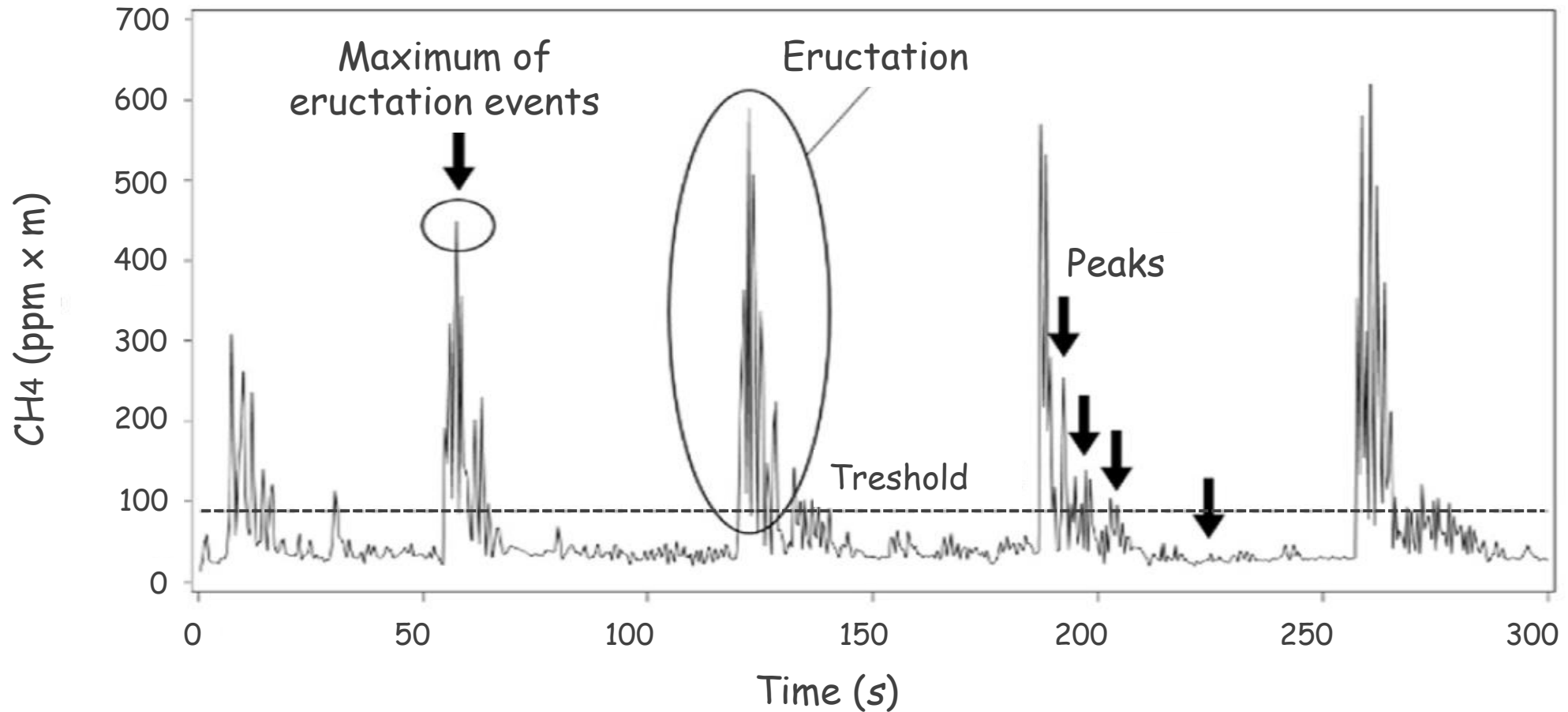


Android app  
Wi-Fi





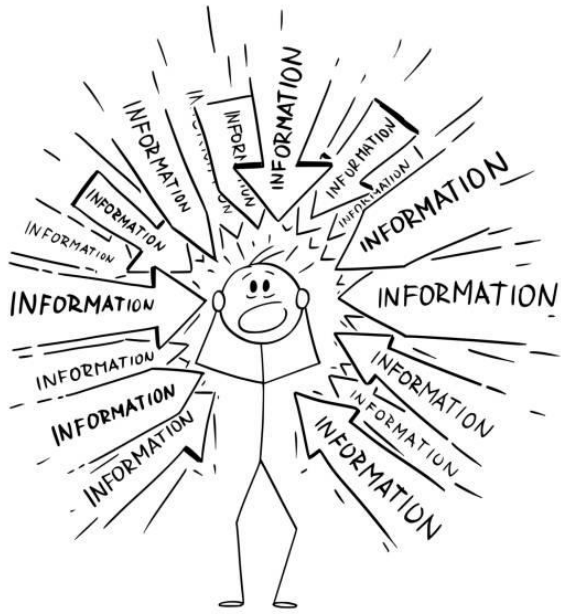




**Figure 1.** Profile of the CH<sub>4</sub> concentration in the breath of a dairy cow as measured with the laser methane detector. The threshold (T) separates eructation and respiration values and is calculated as  $T=Q3 + (1.5 \times (Q3-Q1))$ , with Q1 and Q3 being the first and third quartile of the distribution of all CH<sub>4</sub> values in a profile (Sorg i sar. 2018)

# How to reduce methane emissions from dairy farms?

Because of media/social media there is a huge amount of data - some are scientifically true some not



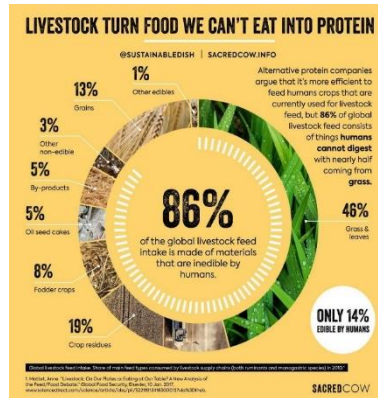
**Data analysis is important.**

Due to huge amount of data there is the possibility for manipulation by intentionally or unintentionally selecting only certain information

# Veganism

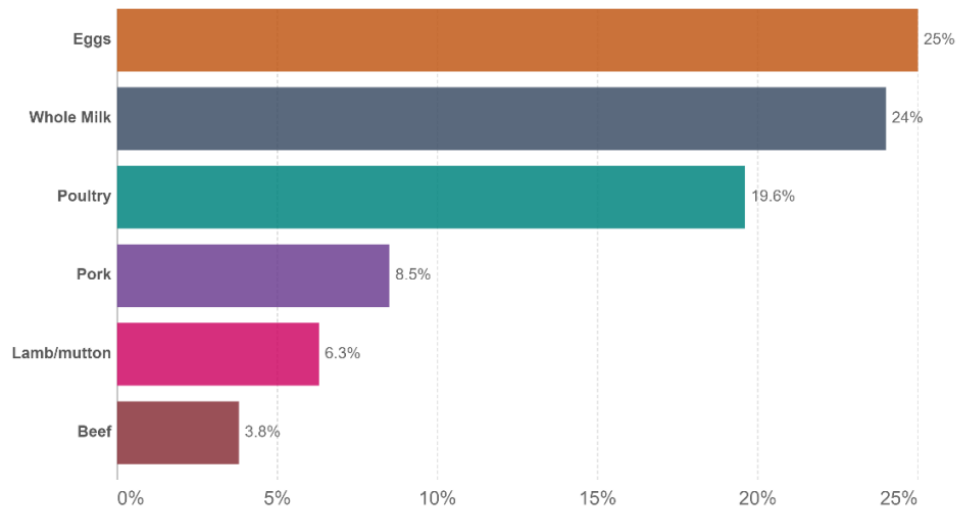
The importance of cows farms as a source of high-quality food for human population

Cows consume food **not edible for humans from land that is unusable to humans and convert it in high-quality food**



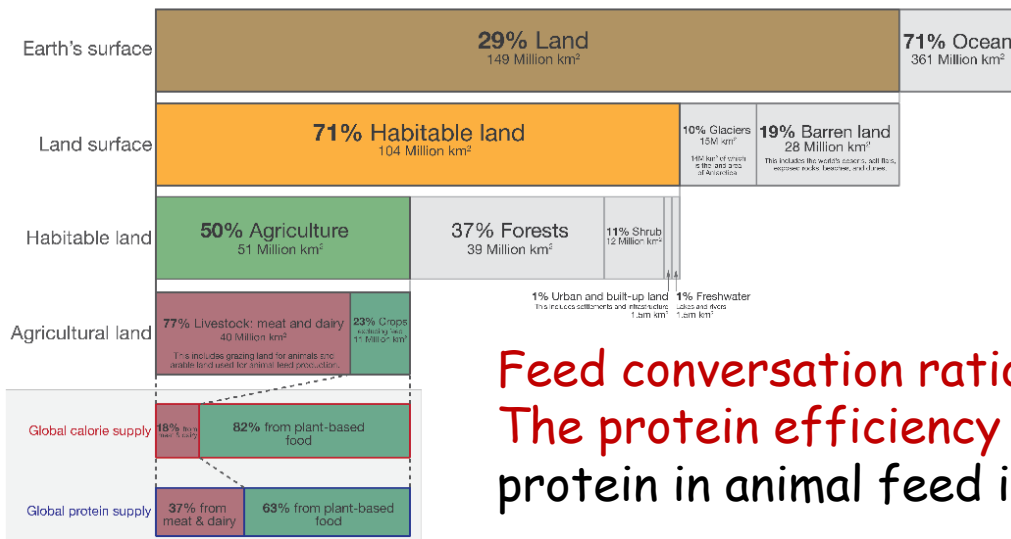
## Protein efficiency of meat and dairy production

The protein efficiency of meat and dairy production is defined as the percentage of protein inputs as feed effectively converted to animal product. An efficiency of 25% would mean 25% of protein in animal feed inputs were effectively converted to animal product; the remaining 75% would be lost during conversion.



Source: Alexander et al. (2016). Human appropriation of land for food: the role of diet. Global Environmental Change. OurWorldInData.org/meat-production • CC BY

## Global land use for food production



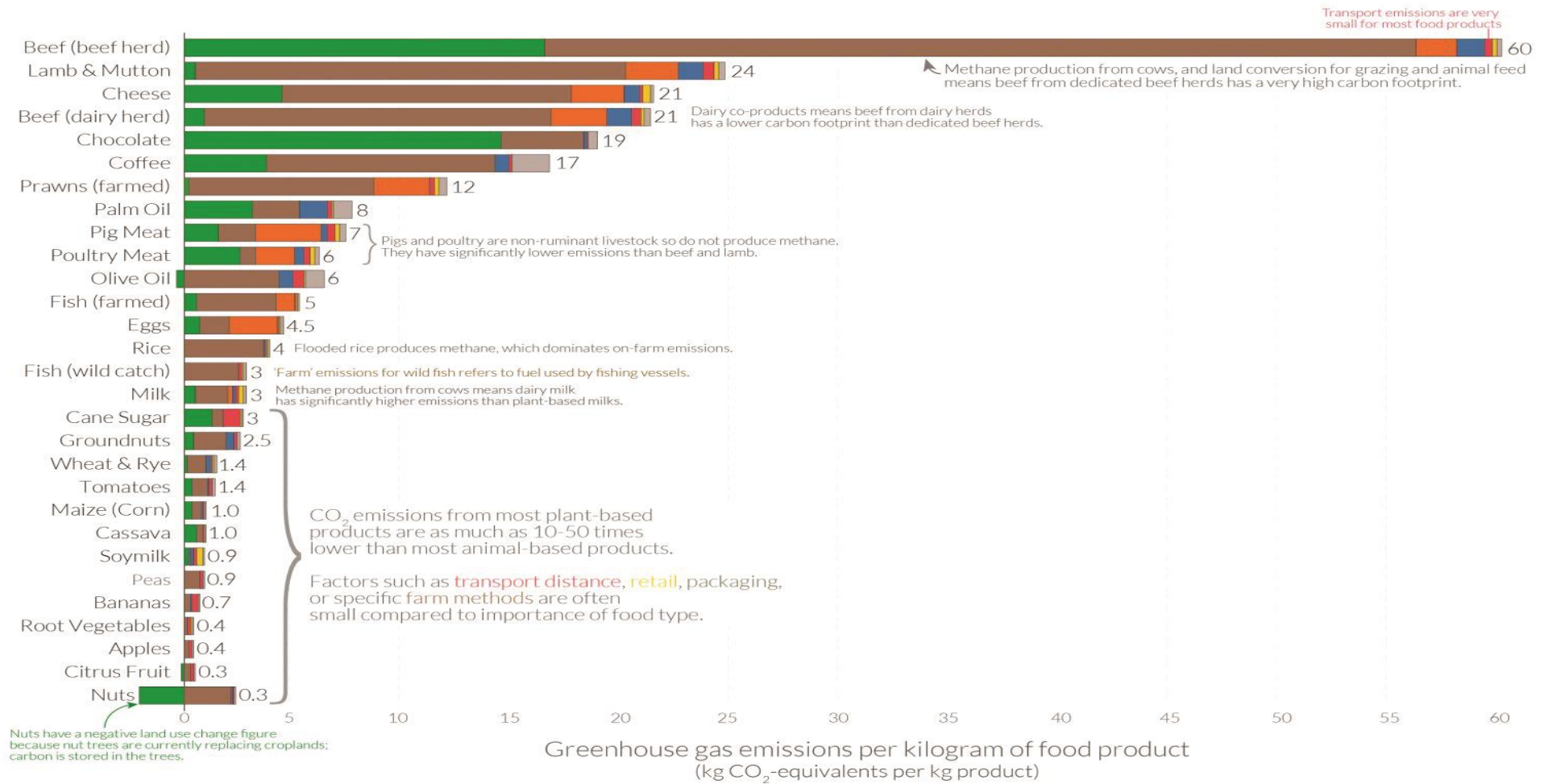
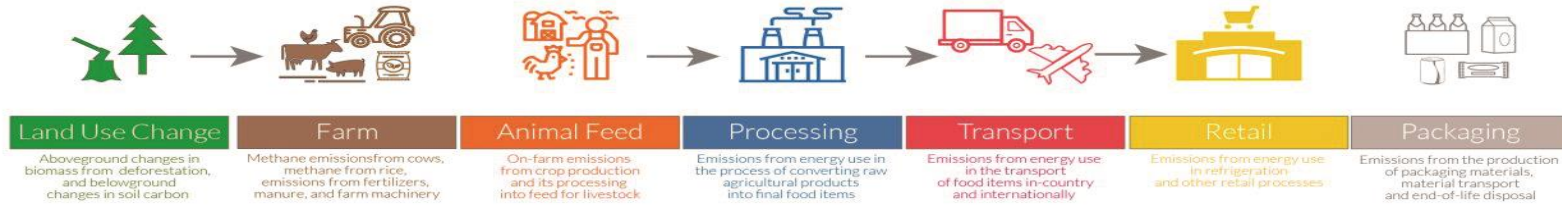
**Feed conversion ratio** is a measure of amount of feed needed to produce milk/meat. **The protein efficiency** of meat/dairy production is defined as the percentage of protein in animal feed inputs as feed efficiently converted to animal products.

Data source: UN Food and Agricultural Organization (FAO) OurWorldInData.org Research and data to make progress against the world's largest problems.

Licensed under CC BY by the authors Elizabeth Birchard and Max Roser in 2019.

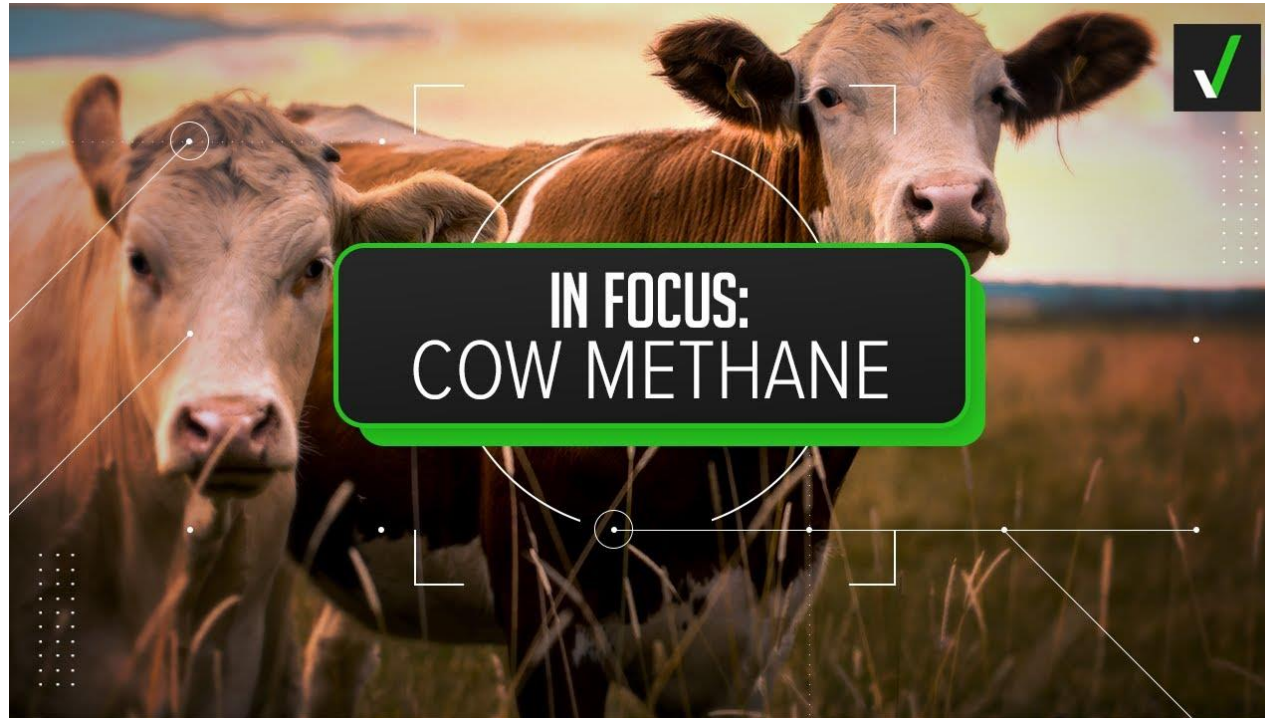
**ANIMAL FEED UNEDIABLE FOR HUMANS**

# Food: greenhouse gas emissions across the supply chain



Note: Greenhouse gas emissions are given as global average values based on data across 38,700 commercially viable farms in 119 countries. Data source: Poore and Nemecek (2018). Reducing food's environmental impacts through producers and consumers. *Science*. Images sourced from the Noun Project. OurWorldinData.org - Research and data to make progress against the world's largest problems. Licensed under CC-BY by the author Hannah Ritchie.

# Veganism



Livestock (21,3%)

**SOURCE OF METHANE**

Rice (6.9%)

# Veganism



Livestock (21,3%)

**SOURCE OF METHANE**

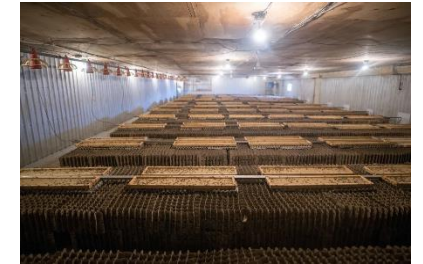
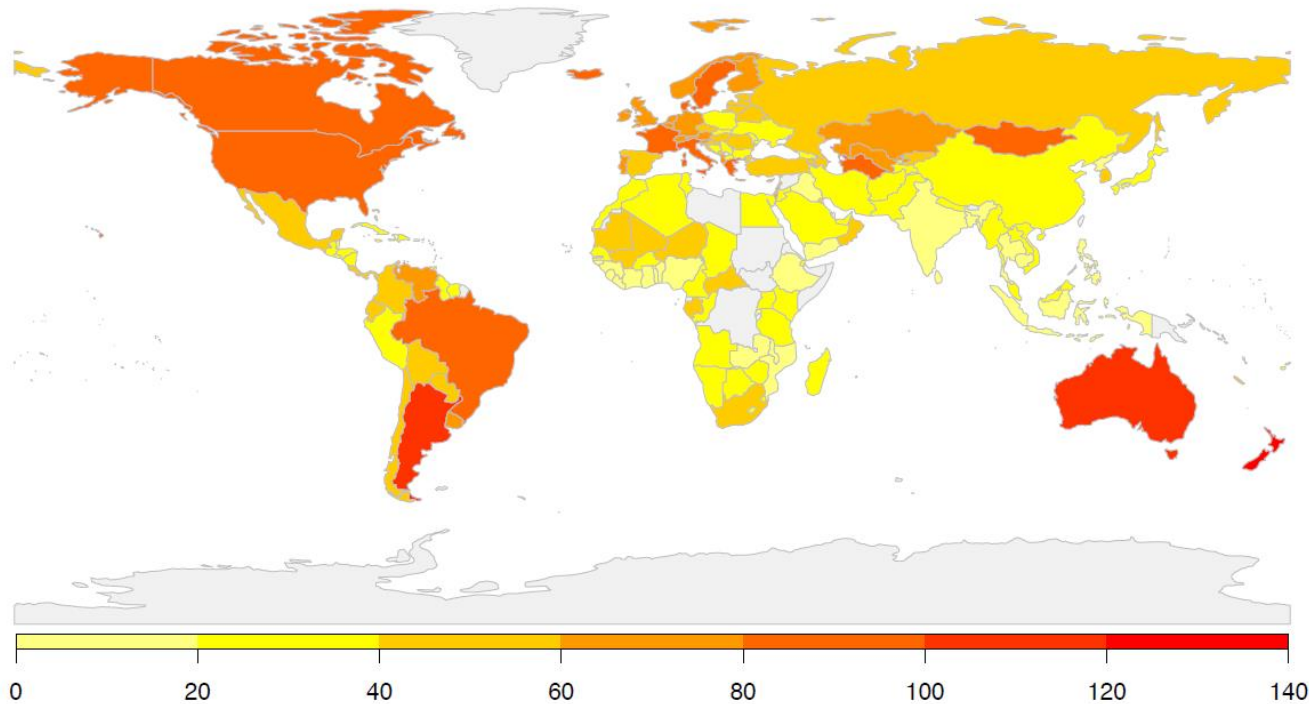
Rice (6.9%)

**If everyone would be vegan there would not be enough food to feed all of humanity  
Cows as plant feed convertors are necessary**

# Changing the type of nutrients used by human

consumption of new nutrients and reduced food elimination

FAOSTAT - the world's largest database of food and agriculture statistics



Source: FAOSTAT, 2015d. Commodity Balances/Crops Primary Equivalent (2015-12-16). Food and Agriculture Organization of the United Nations, Rome, Italy.

**HALF (Human Appropriation of Land for Food) index** - How much land has been appropriated for feeding humanity.  
average: 35.1 (0.65 ha of land per person)

**USA: 97.7/India: 15.8**

**If all humanity adapt to nutrition obtain by USA citizens: 178% more of agriculture land will be needed**

**If all humanity adapt to nutrition obtain by Indian citizens: 55% less of agriculture land will be needed**



# Manure Management

Storage of faeces in anaerobic conditions allows the growth of methanogens.

## HYDROLISIS-

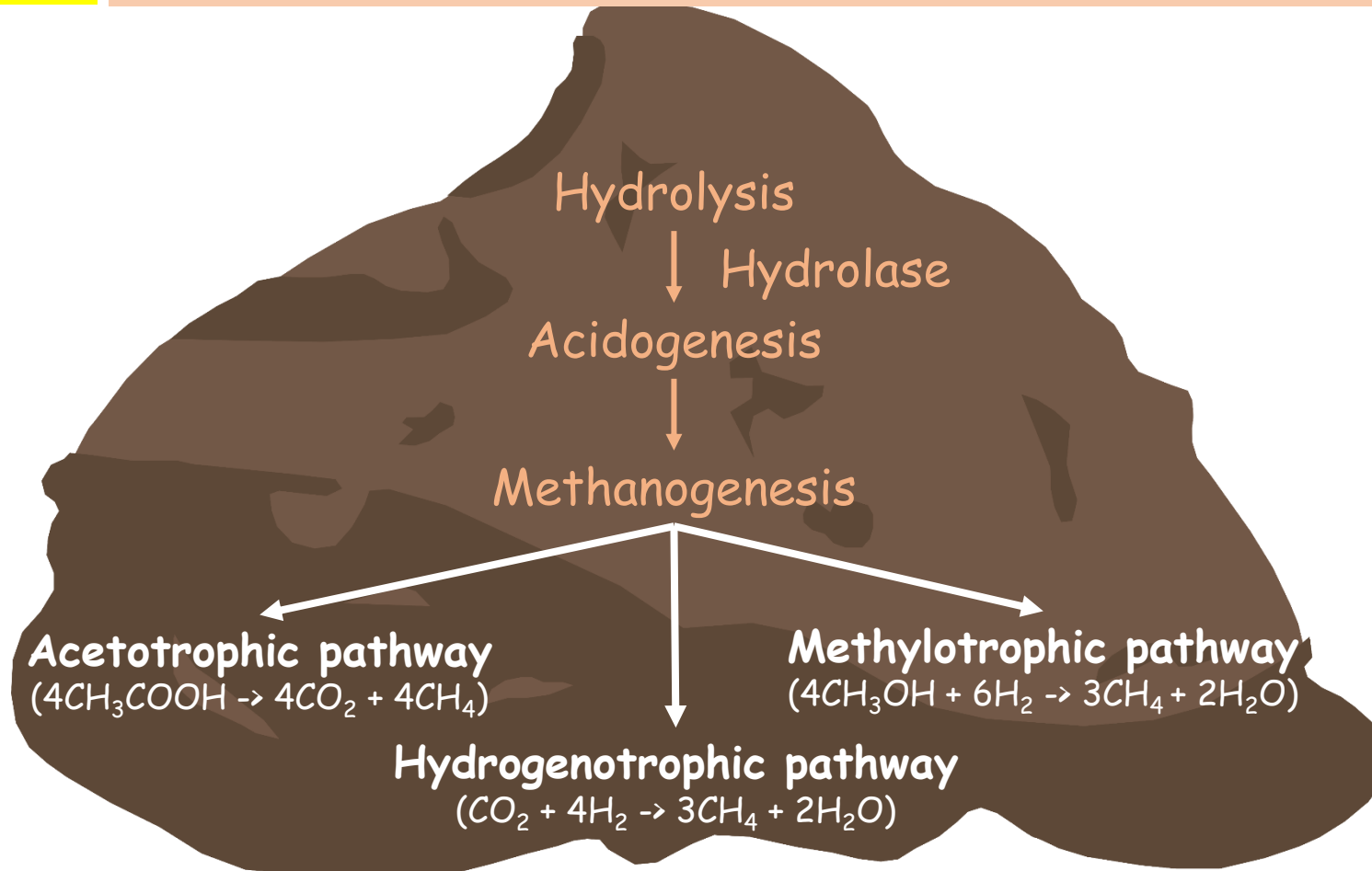
breakdown fat, protein, CH to long-chain FA and simple sugars

**ACIDOGENESIS-** conversion of simple compounds into short-chain FA (lactic, propionic, butiric) used by homoacetogenic mo that produce acetic acid and release  $CO_2$  and  $H^+$  - starting compounds for methanogenesis

~~$O_2$~~



Methanogens



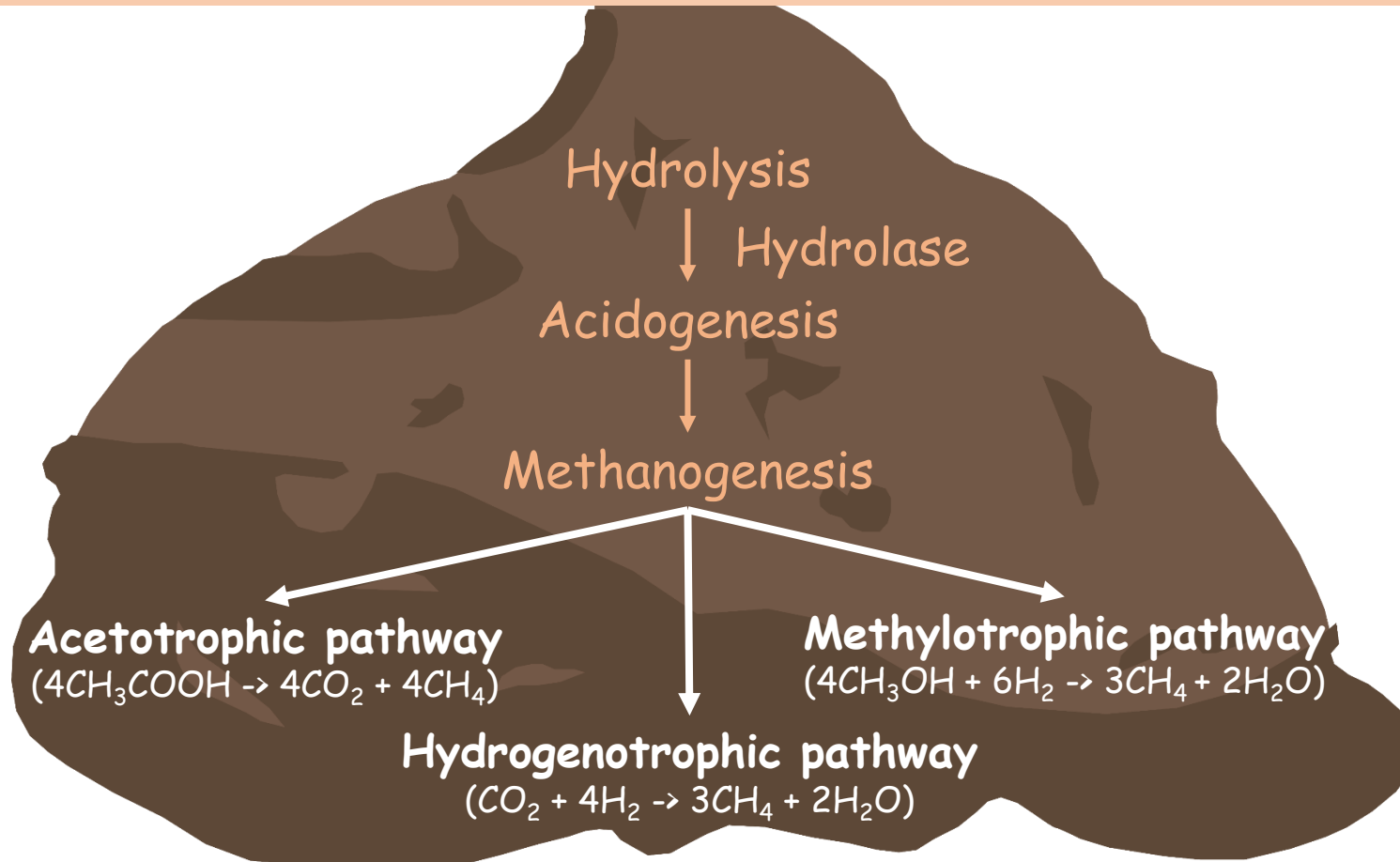
# Manure Management

Storage of faeces in anaerobic conditions allows the growth of methanogens.

**METHANOGENES**- occurs in one of three pathways. Acetotrophic pathway is dominant. Emission of methane from manure participate approximately 2% in antropogenic emission. The amount released depends on manure management (humidity, pH, temperature), season (ambient temperature), manure composition



Methanogens



**Manure management** - Manure stored anaerobically - higher CH<sub>4</sub> production

**Manure temperature control** - 1-2°C ↓ CH<sub>4</sub> 5-10% ↓

**Acidification of manure** -

pH 7 optimum  
pH 6.5/pH 8.3; ↓CH<sub>4</sub> 50%; pH 4.5 NH<sub>4</sub> i N<sub>2</sub>O ↓

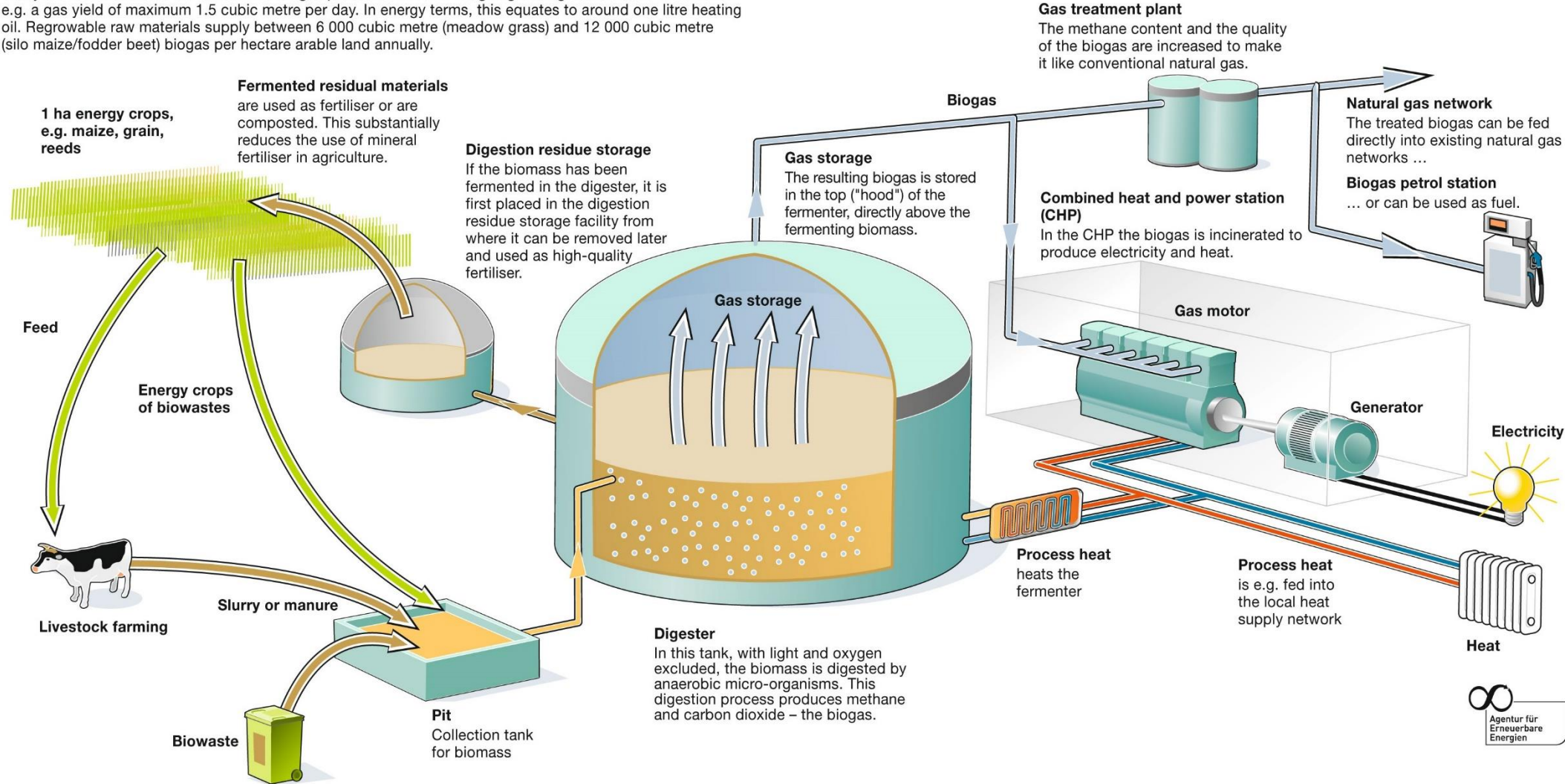
Nitric acid  
Citric acid  
Lactic acid



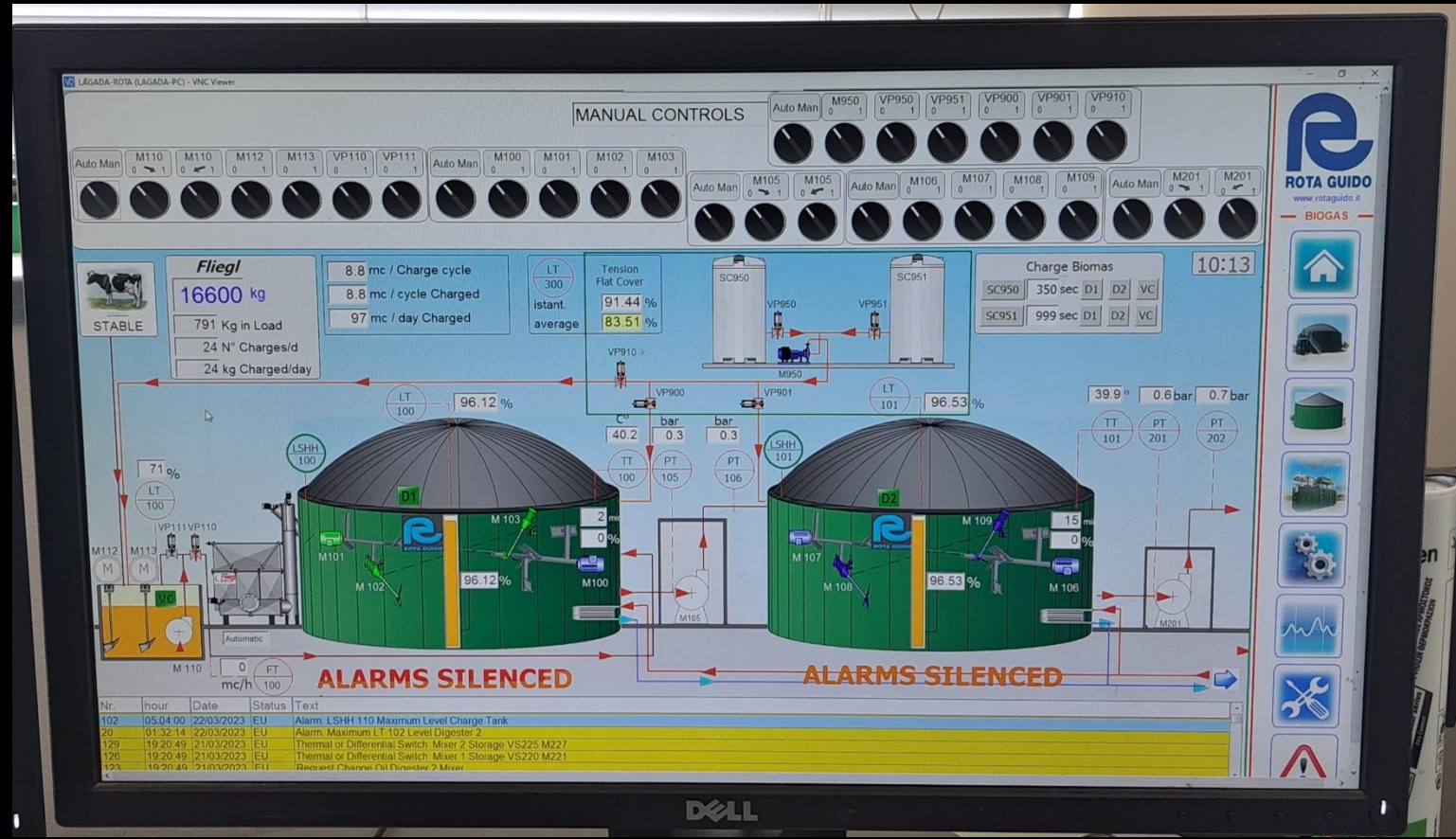
# Anaerobic digestion can be used for the production of biogas in special plants

## Biogas system

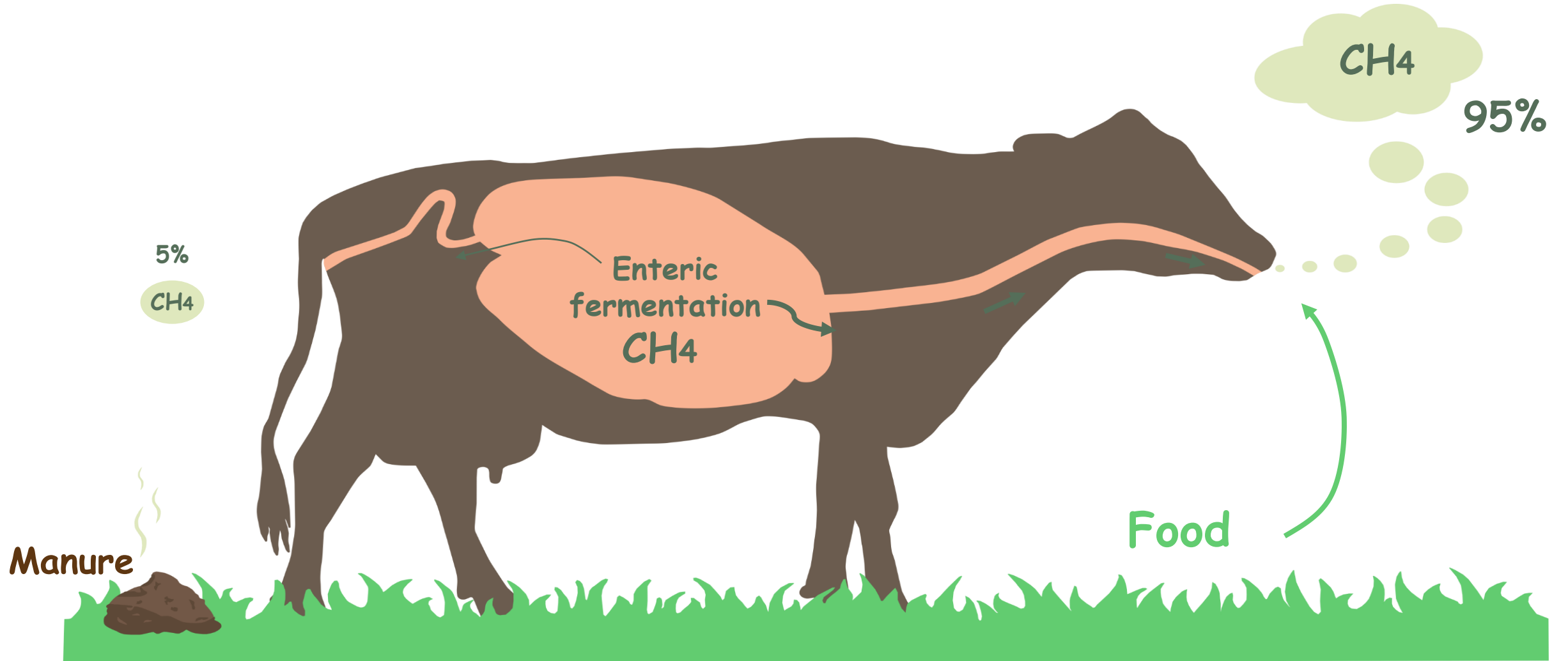
Slurry and solid biomass are suitable for biogas production. A cow weighing 500 kg can be used to achieve e.g. a gas yield of maximum 1.5 cubic metre per day. In energy terms, this equates to around one litre heating oil. Regrowable raw materials supply between 6 000 cubic metre (meadow grass) and 12 000 cubic metre (silo maize/fodder beet) biogas per hectare arable land annually.







# Nutritive Modulation of Cow's Metabolism



A diagram of a rumen cross-section, showing a large, irregularly shaped chamber. The top part is a lighter pinkish-red color, and the bottom part is a darker, more textured pinkish-red color. The bottom part contains a dense layer of green, fibrous material representing feed. The text is overlaid on the diagram in various colors and sizes.

Rumen is fermentation chamber

Rumen homeostasis

High humidity (water and saliva)

Abundance of nutrients

Anaerobic condition

Osmolarity (260-340 mmol/L)

Temperature (37-42 °C)

pH (6-6.5)



# Types of bacteria in the rumen

## Based on their substrate



Celullolytic

Hemicellulolytic



Amylolytic

Pectinolytic

Proteolytic

Urealytic

## Based on their products

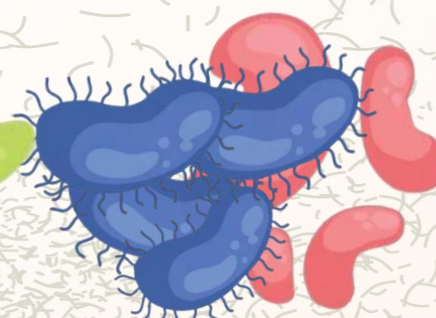
**Methanogenic**

(phylum Euryarcheota, domein of Archaea)  
Archaea-differs from eucaryotes and similar to bacteria with their own cofactors (coenzymes M, F420 and F 430) and lipids (isoprene-glycerol esters)

Ammonia-producing



Protozoa



Bacteria



Fungi

## Based on consumption

Sugar consuming

Acids consuming

Lipid consuming

**Macromolecules**  
(Carbohydrates)

Primary  
bacteria

**Simple molecules**  
(monosaccharides - **GLUCOSE**)

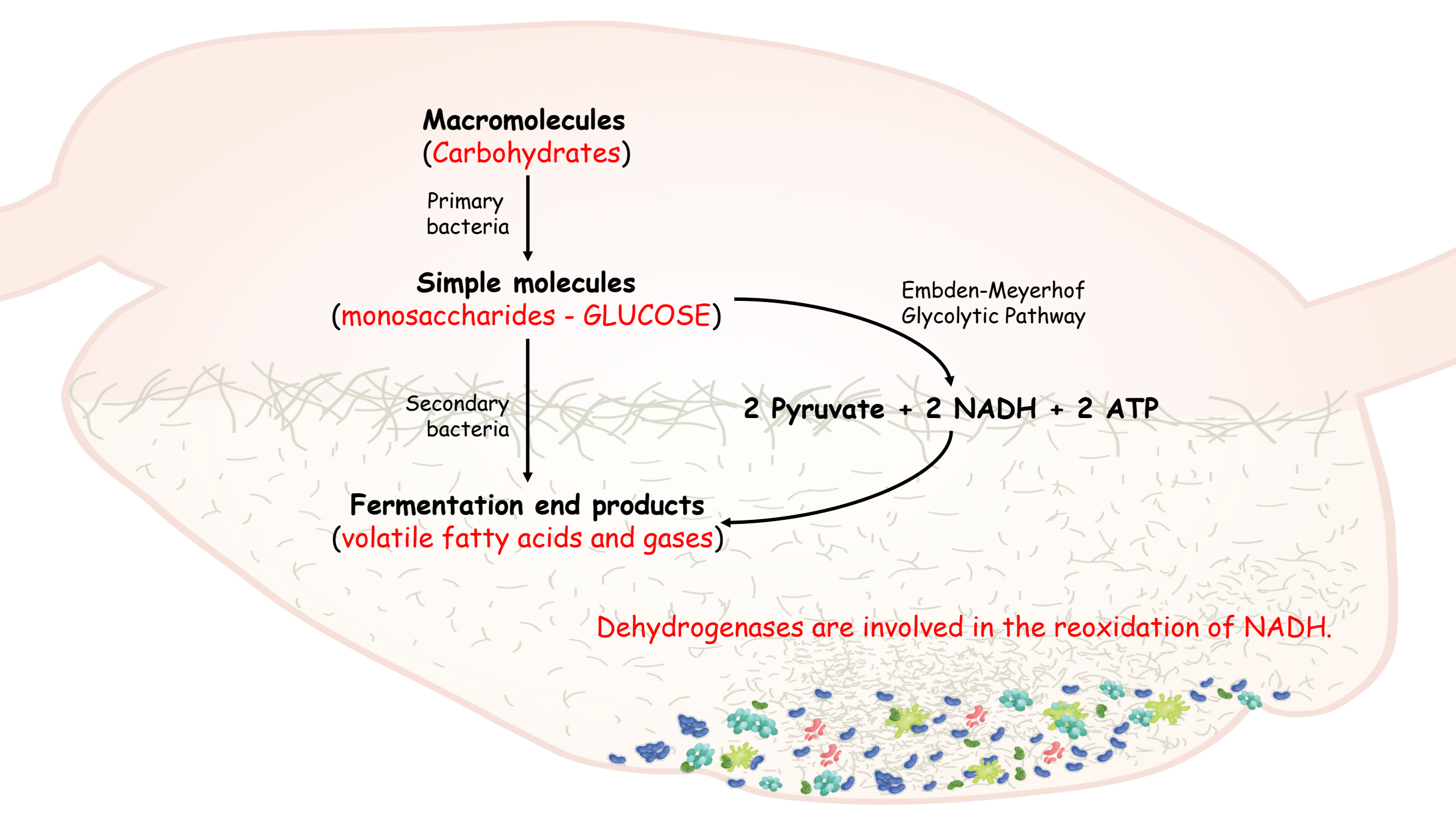
Embden-Meyerhof  
Glycolytic Pathway

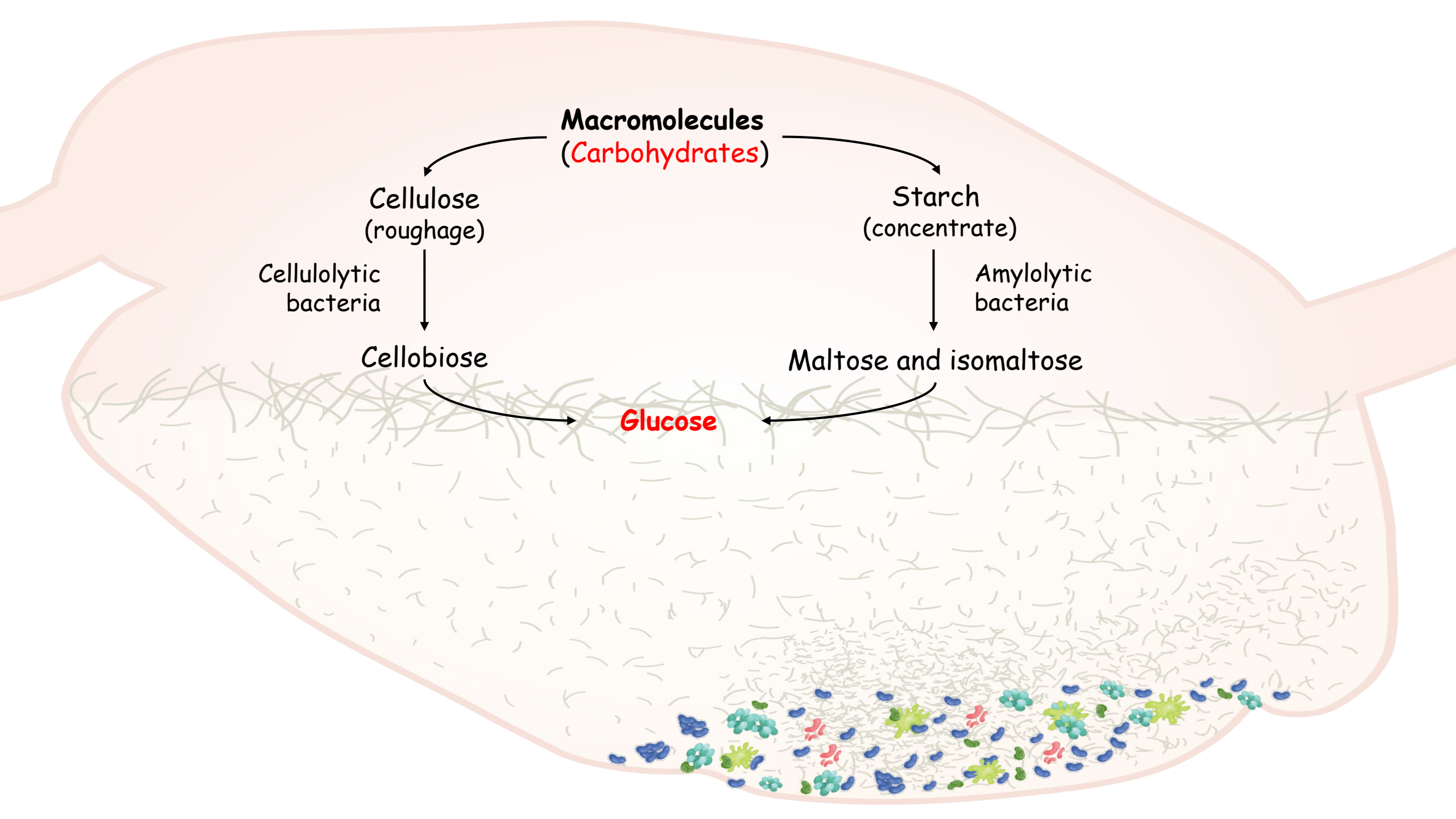
Secondary  
bacteria

**2 Pyruvate + 2 NADH + 2 ATP**

**Fermentation end products**  
(volatile fatty acids and gases)

Dehydrogenases are involved in the reoxidation of NADH.





**Macromolecules  
(Carbohydrates)**

Cellulose  
(roughage)

Starch  
(concentrate)

Cellulolytic  
bacteria

Amylolytic  
bacteria

Cellobiose

Maltose and isomaltose

**Glucose**

2 Acetate

$CO_2$

$CO_2$

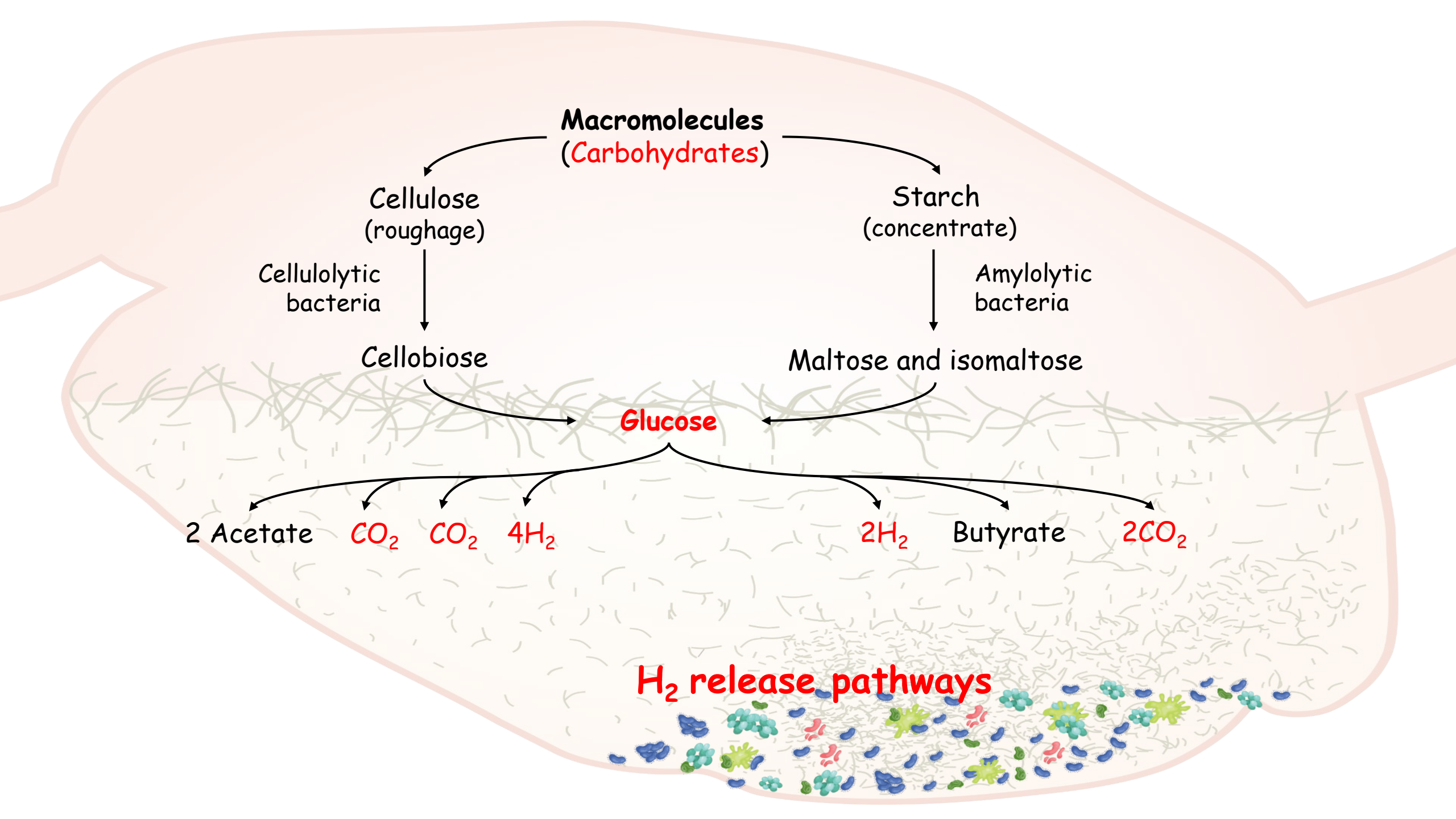
$4H_2$

$2H_2$

Butyrate

$2CO_2$

**$H_2$  release pathways**



**Macromolecules  
(Carbohydrates)**

Cellulose  
(roughage)

Cellulolytic  
bacteria

Cellobiose

Starch  
(concentrate)

Amylolytic  
bacteria

Maltose and isomaltose

**Glucose**

2 Acetate

$CO_2$

$CO_2$

$4H_2$

$CH_4$

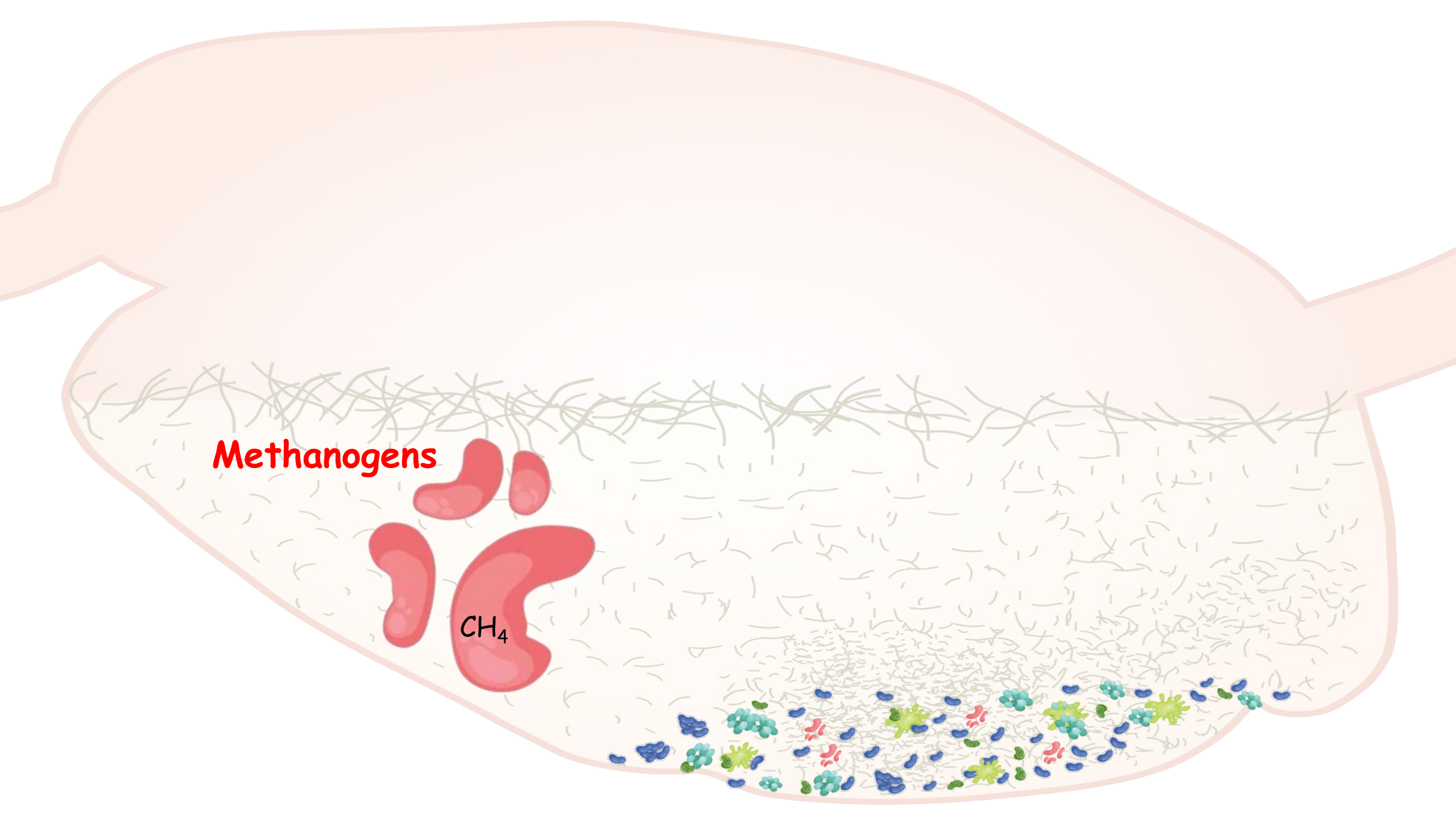
$2H_2$

2 Propionate

Butyrate

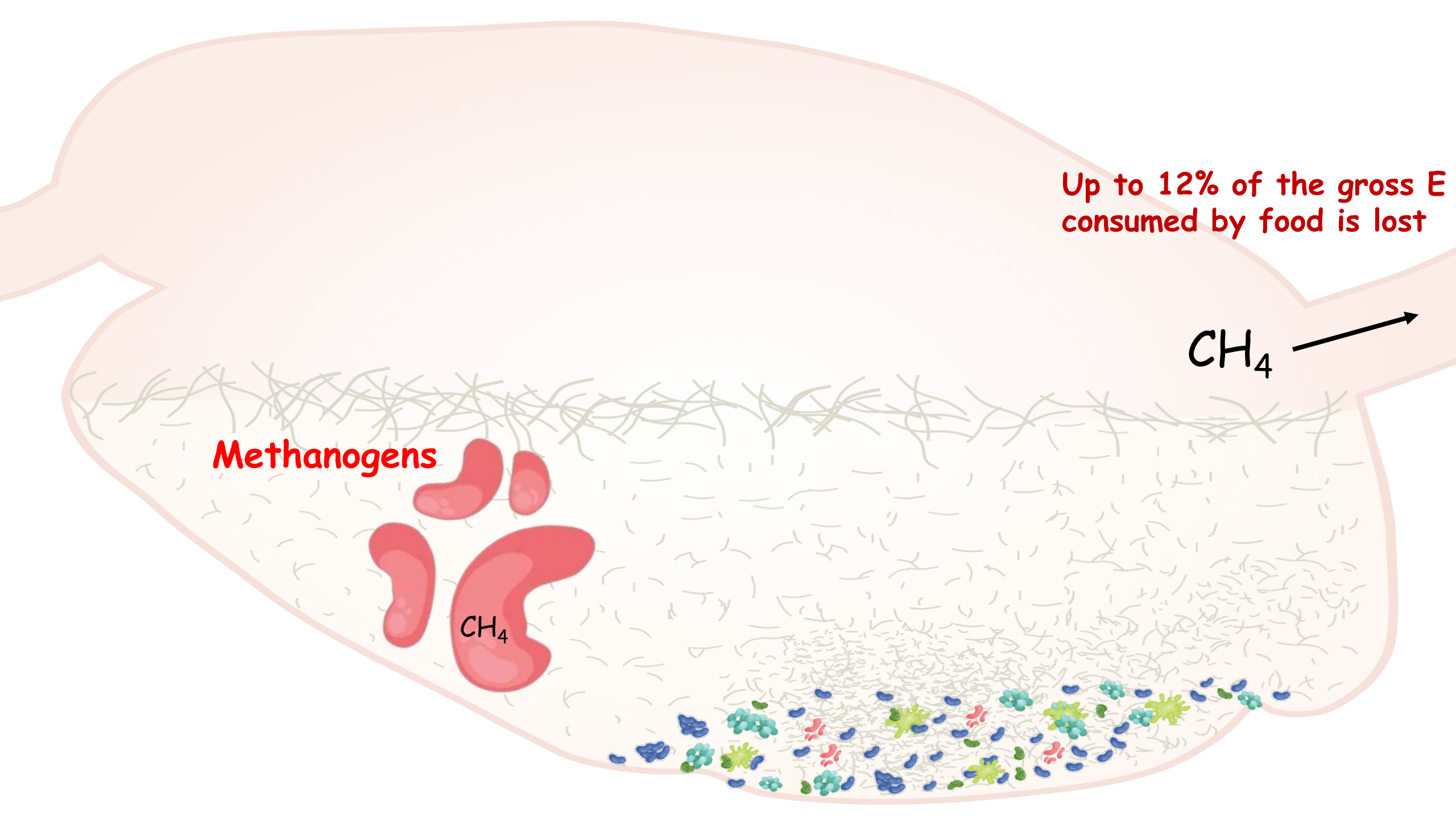
$2CO_2$

**$H_2$  consumption pathways**



**Methanogens**

CH<sub>4</sub>

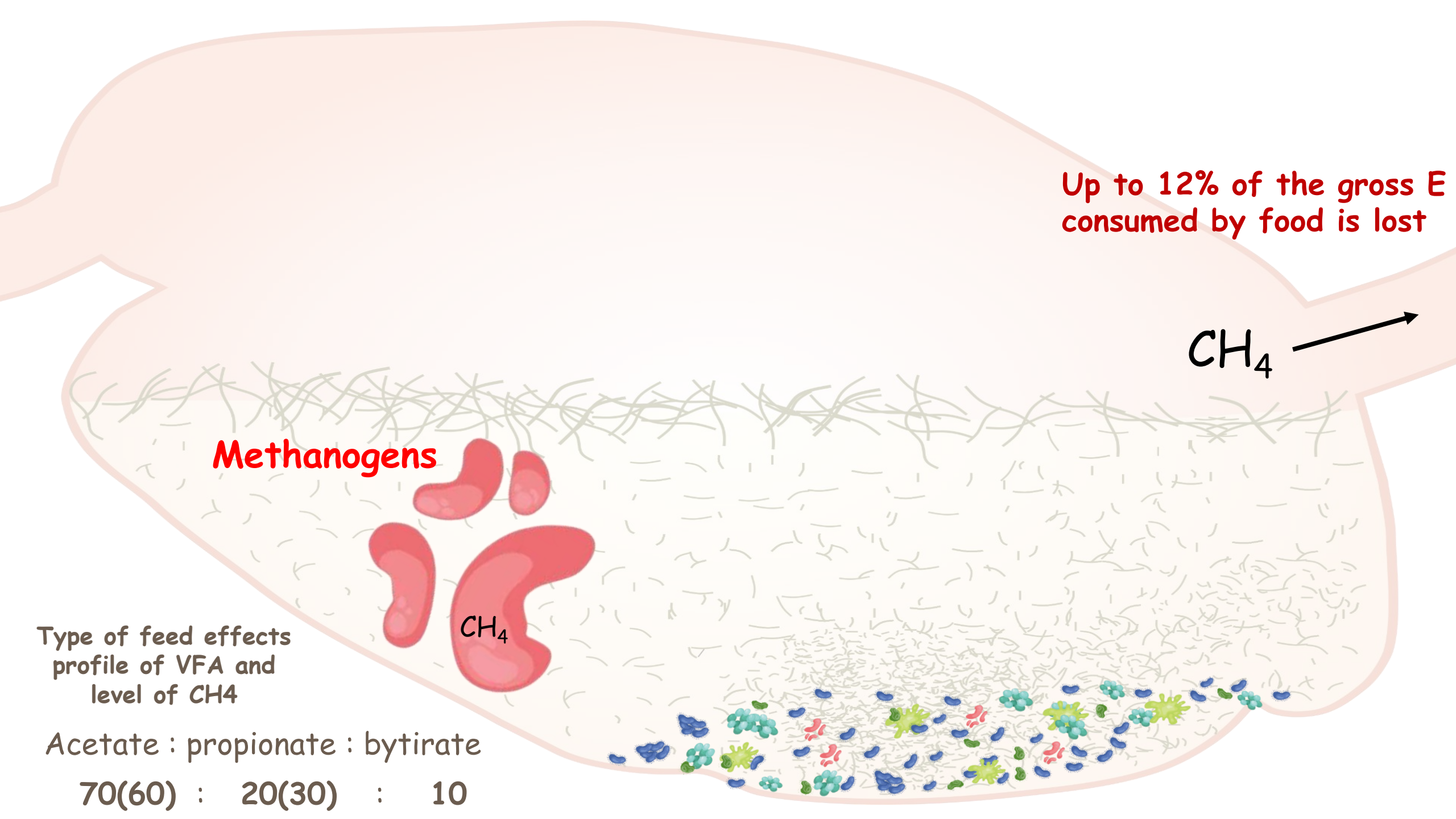


Up to 12% of the gross E consumed by food is lost

CH<sub>4</sub> →

Methanogens

CH<sub>4</sub>



Up to 12% of the gross E consumed by food is lost

CH<sub>4</sub> →

**Methanogens**

CH<sub>4</sub>

Type of feed effects  
profile of VFA and  
level of CH<sub>4</sub>

Acetate : propionate : butyrate  
70(60) : 20(30) : 10



**CH<sub>4</sub> production in rumen is essential for the rumen homeostasis** because it avoid excessive accumulation of H<sub>2</sub> which could inhibit the activity of dehydrogenases involved in the reoxidation of reduction equivalents (NADH) formed in previous degradable processes

**Propionate** is end product of rumen fermentation that is probably the principal alternative of the H<sup>+</sup> consumption after CH<sub>4</sub>

**Increases in propionate formation is strongly associated with decreases in CH<sub>4</sub> production**

## Two key groups of methanogens

*Methanobrevibacter* SGMT  
(McrI and McrII)

*Mbb. smithii*, *Mbb. gottschalki*, *Mbb. millerae* i *Mbb. thaueri*

*Methanobrevibacter* RO (due to coenzymes-not so important)  
(McrII)

*Mbb. ruminantium* i *Mbb. olleyae*

CH<sub>4</sub>

Loss of 12% energy

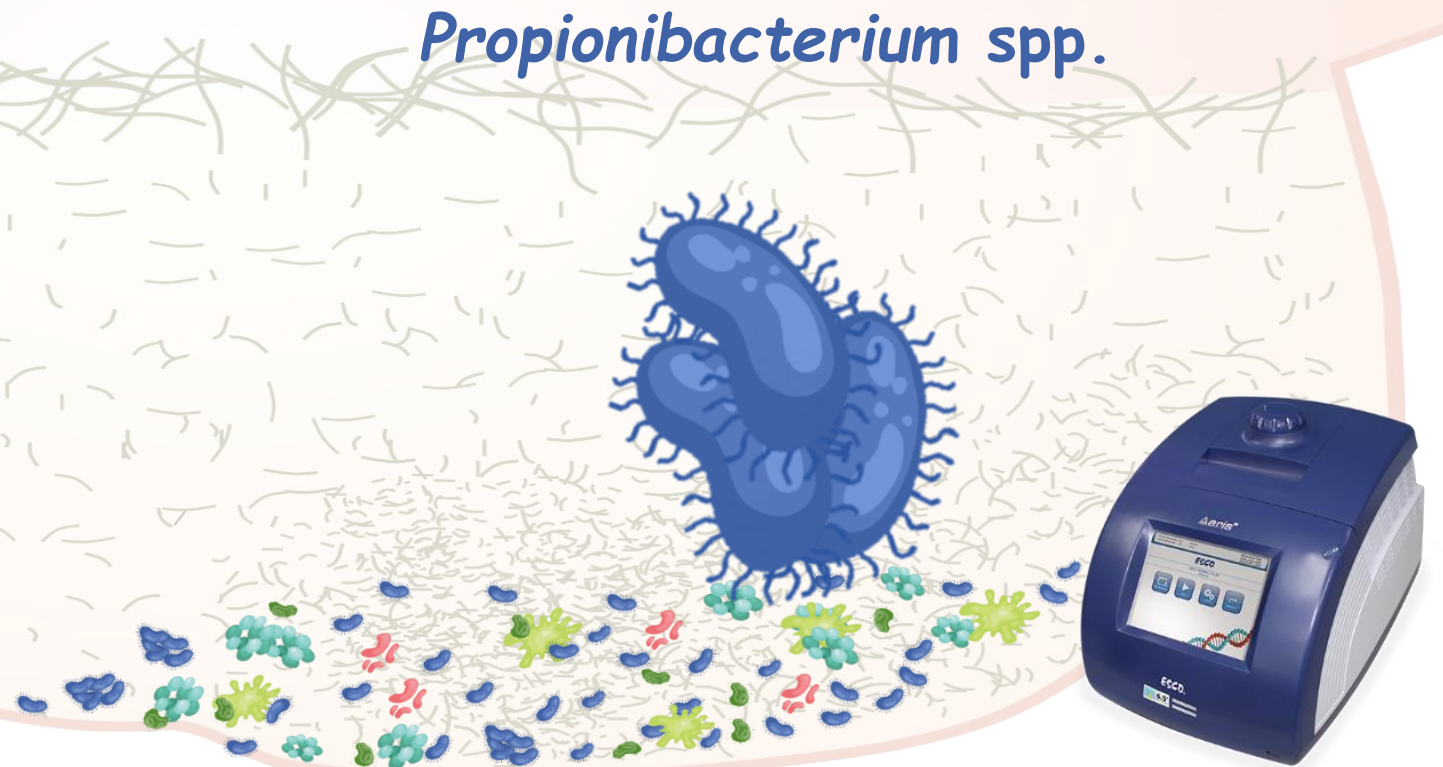
*Propionibacterium* spp.

Methanogens

< 70% E

Acetate : propionate : butyrate

70 : 20 : 10



## Two key groups of methanogens

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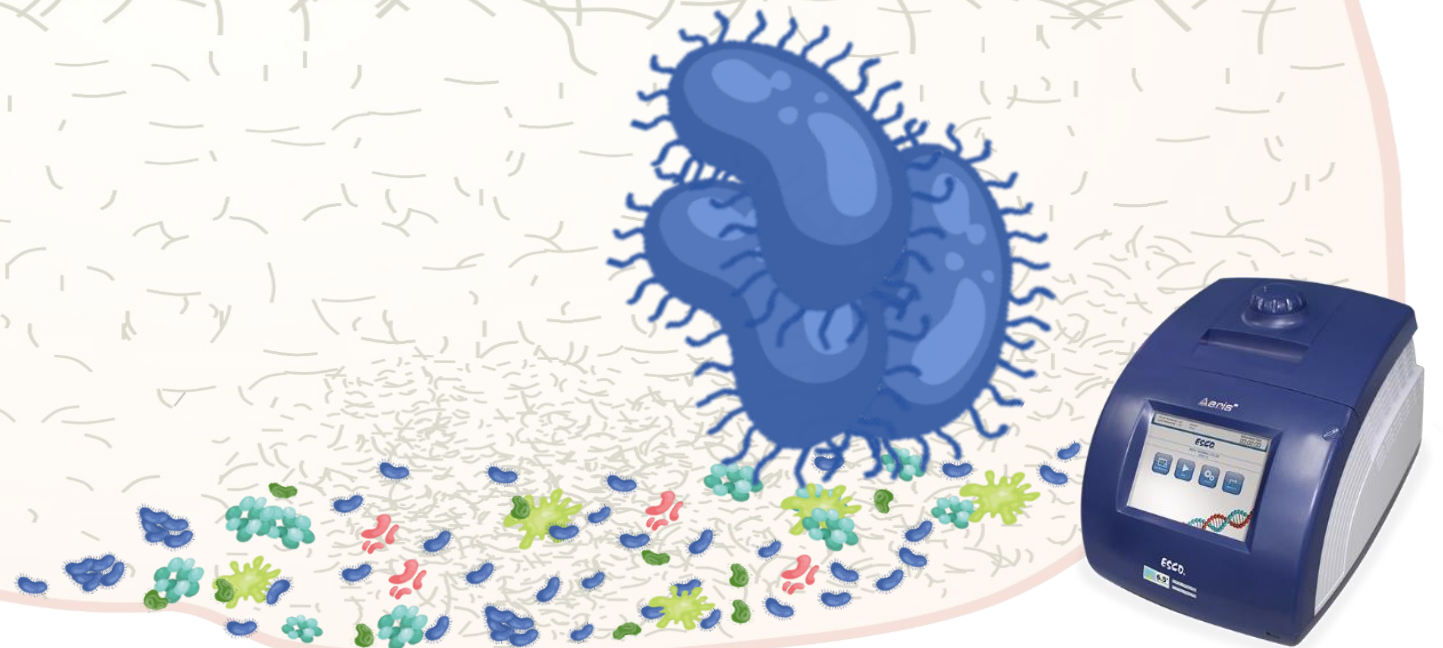
**CH<sub>4</sub>**  
Loss of 12% energy

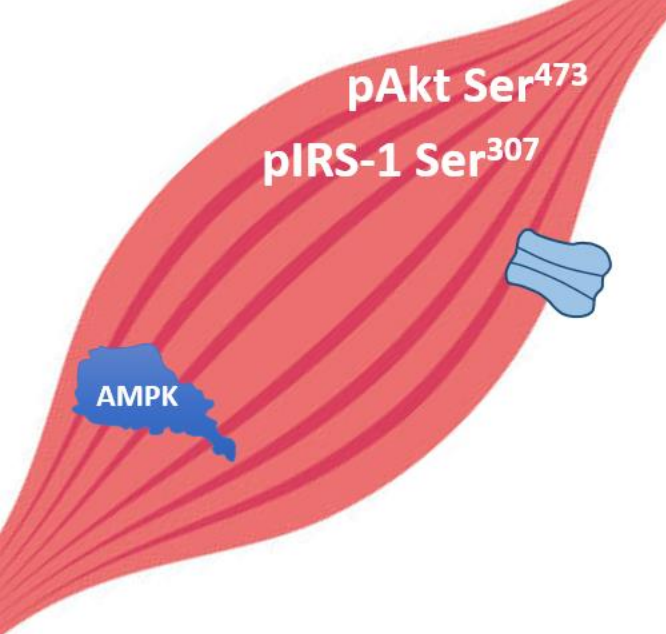
**Methanogens**

*Propionibacterium* spp.

< 70% E

Acetate : propionate : butyrate  
40 : 40 : 20



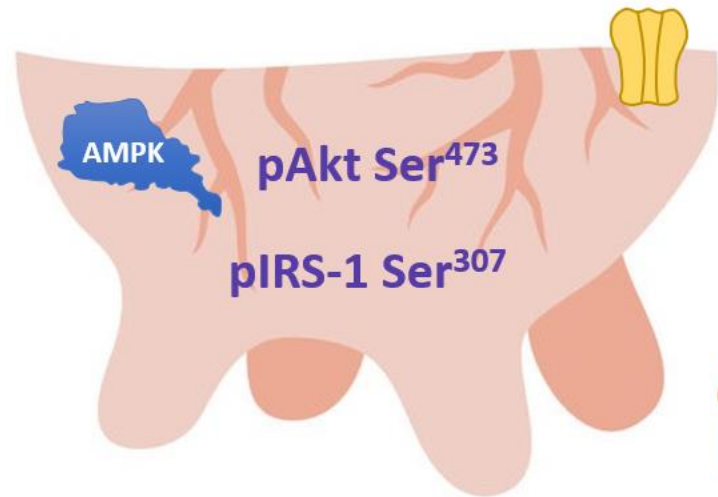


## Muscle tissue (insulin-dependent)

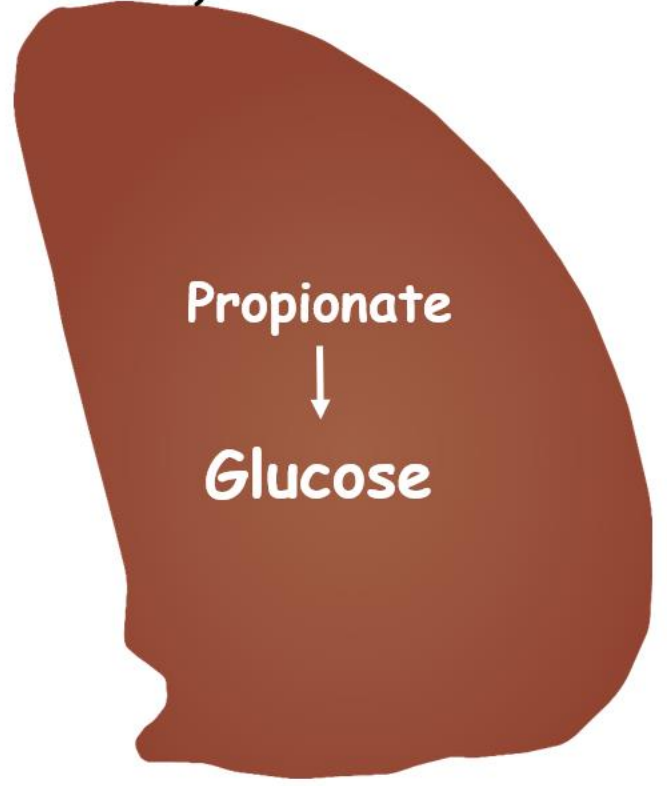
Maximizing the flow of metabolic hydrogen in the rumen away from CH<sub>4</sub> and toward propionate would increase the efficiency of ruminant production.

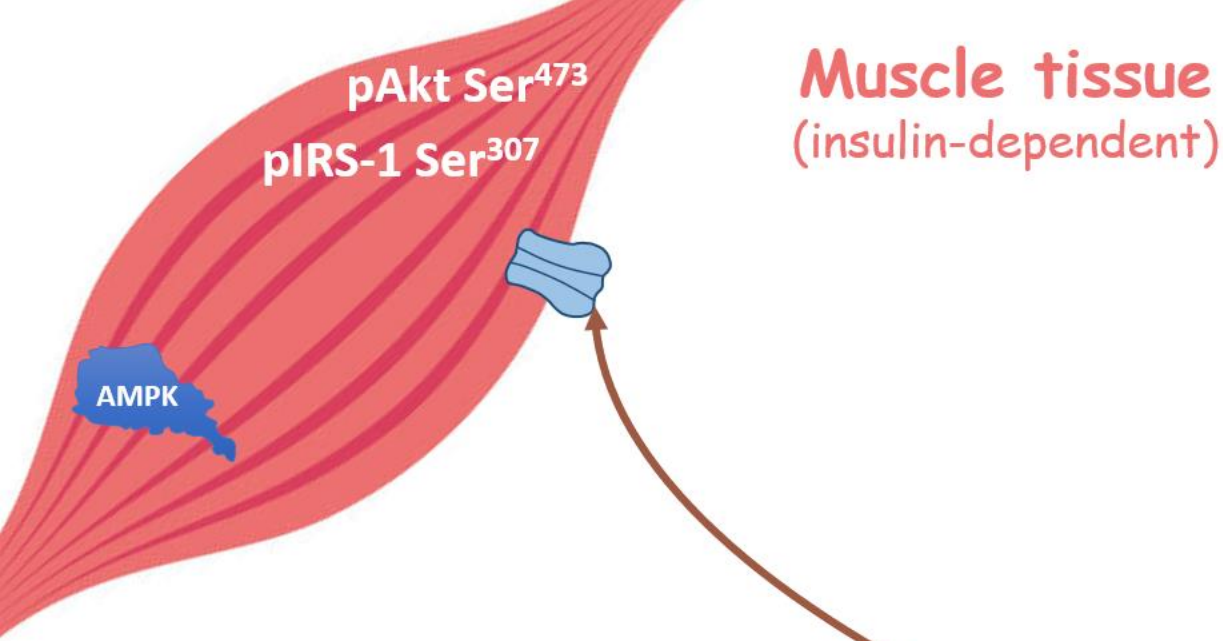
WHY?

Propionate is predominantly used as a glucose precursor in ruminants. From liver, glucose can be redirect to peripheral or mammary tissue (favorable).



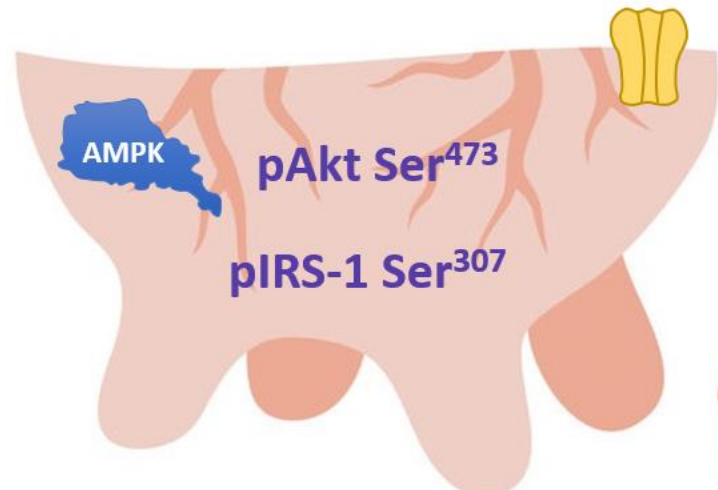
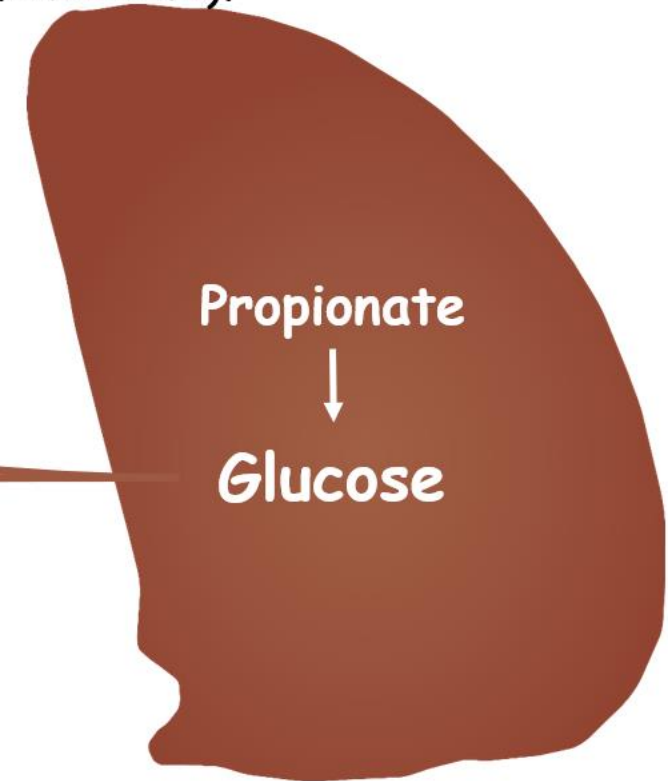
## Mammary gland (insulin-independent)





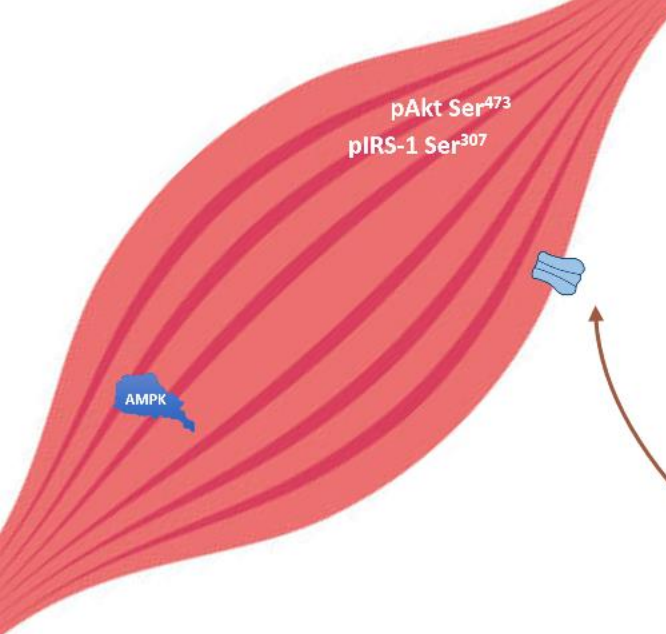
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**Mammary gland**  
(insulin-independent)





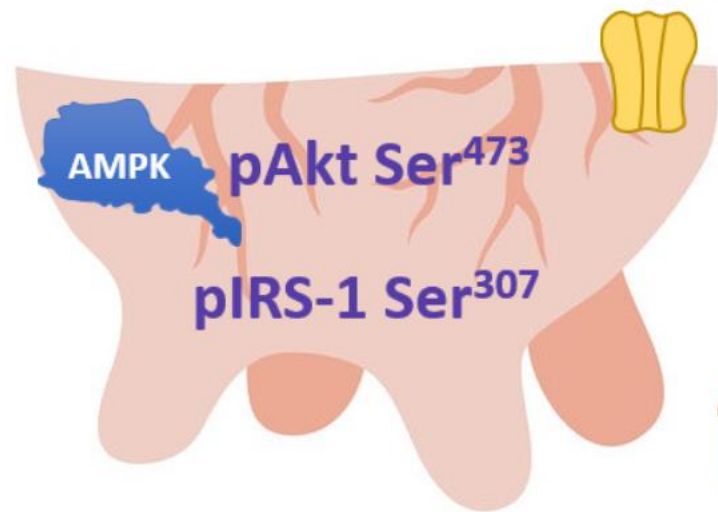
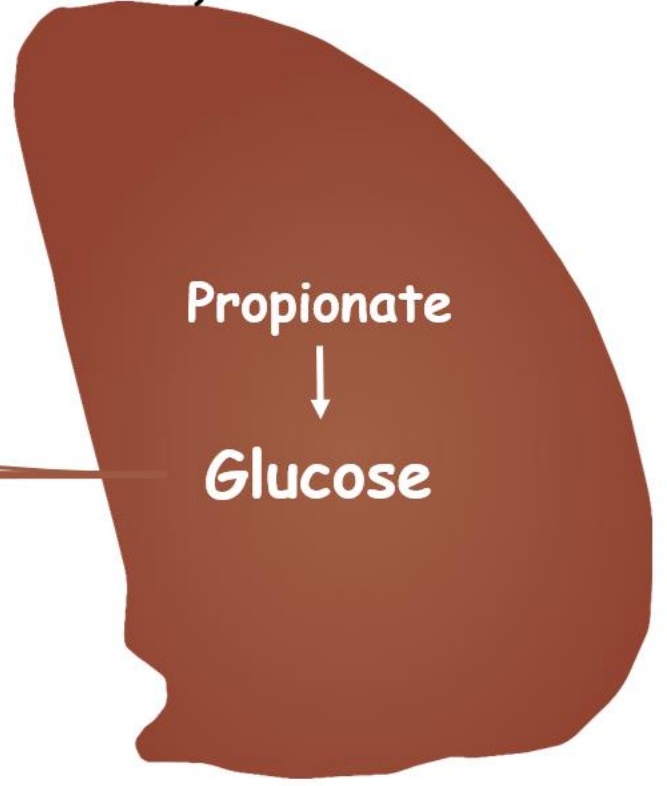
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pAkt Ser<sup>473</sup>  
pIRS-1 Ser<sup>307</sup>

AMPK



AMPK

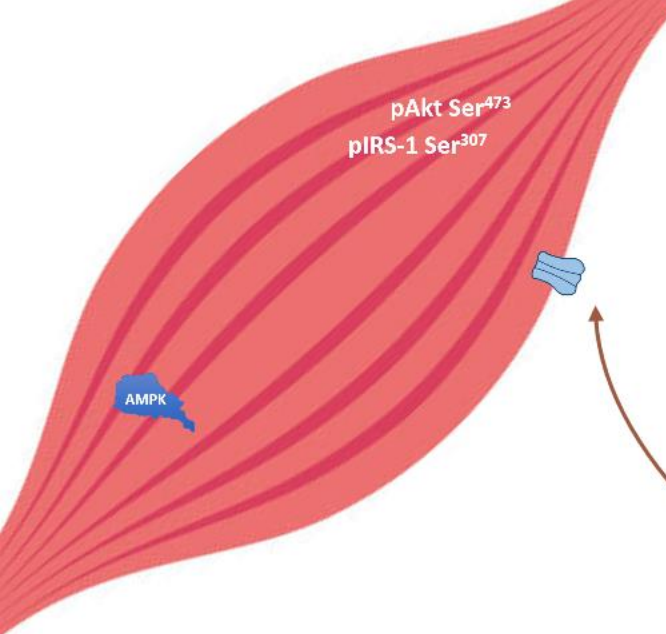
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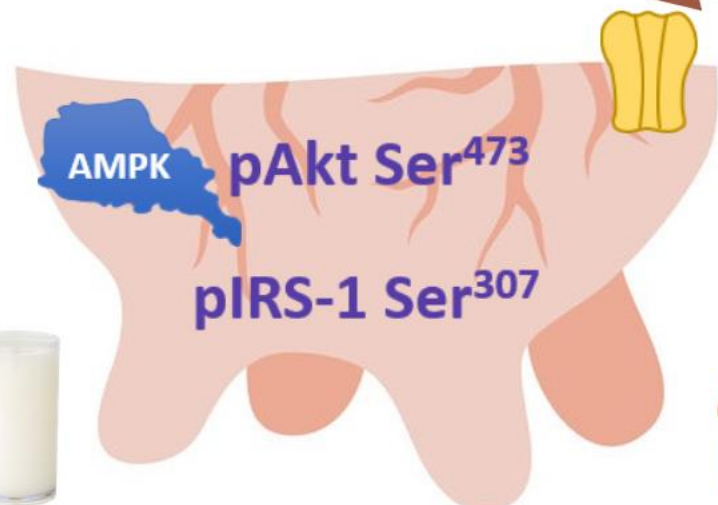
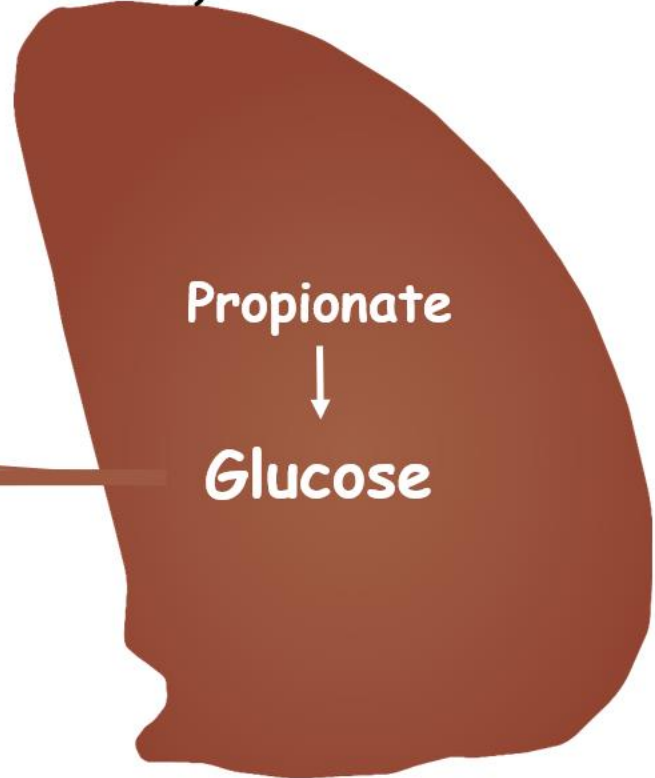




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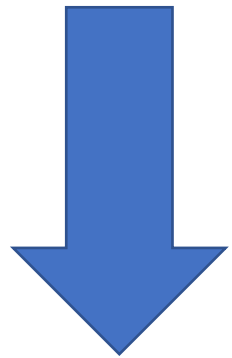


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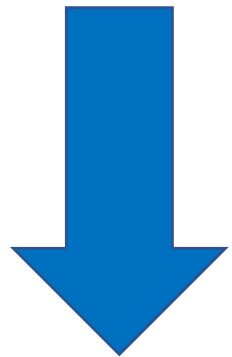


# MAIN GOAL of nutritive modulation of metabolism

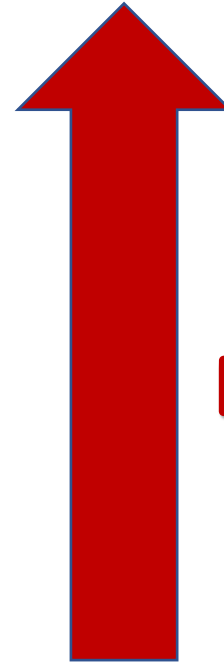
Modifying the dietary formulation to shift the H<sup>+</sup> flow toward alternative electron acceptors, as propionate



CH<sub>4</sub>



Loss of gross  
energy intake



Milk production

Farm profitability

**Ecologically friendly and high-profitable dairy farms**



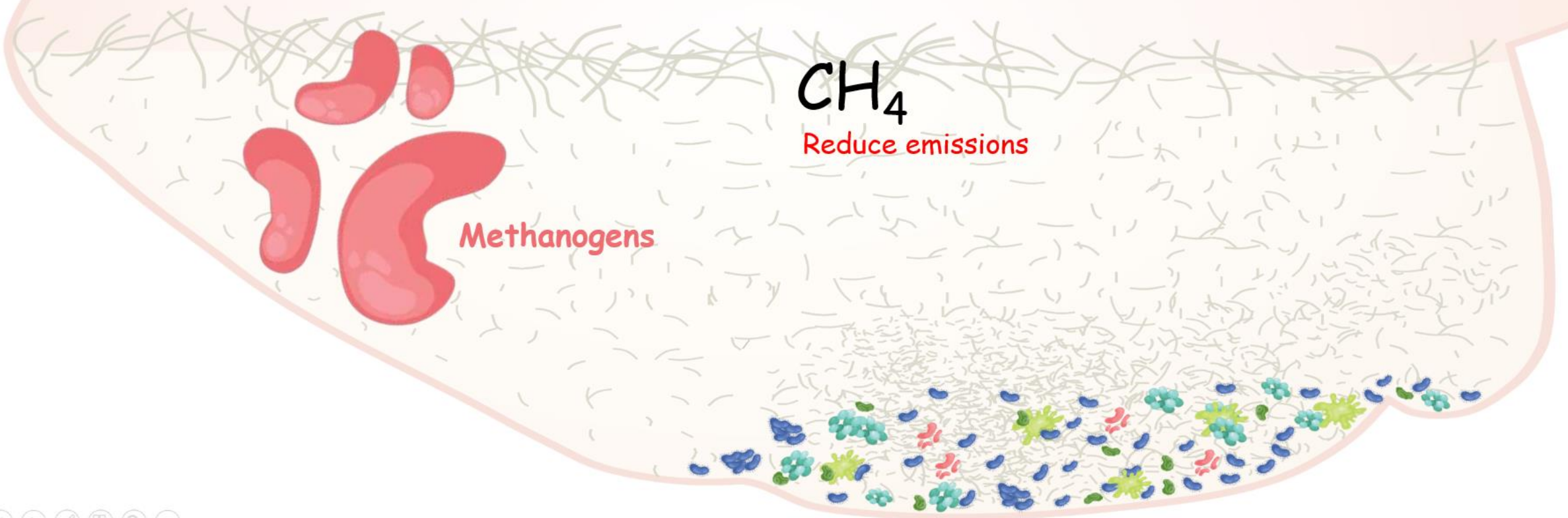
## Nutritive modulation of metabolism Dietary supplements



### Tannins

Bactericidal effect - reducing the population of methanogens (Honan et al., 2021).

Improve antioxidant defense mechanisms (Prodanović et al., 2023).



## Nutritive modulation of metabolism Dietary supplements



Tannins



Diallyl disulfide

Inhibition of the enzyme system of archaea  
(Kirovski et al. - unpublished data).

Antimicrobial properties (Nakamoto et al., 2020).

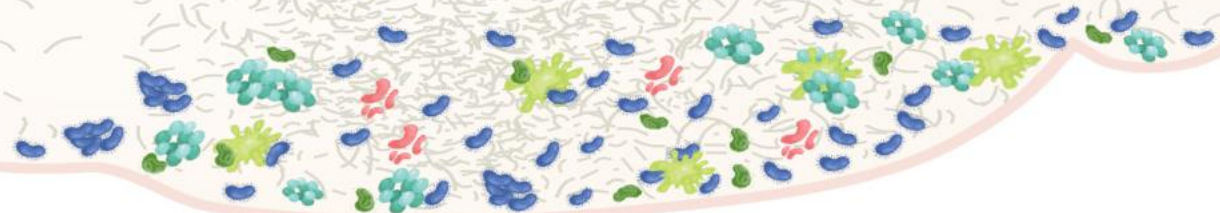
Repellent effect on insects - natural flies control for cattle (Showler, 2017).



Methanogens

$\text{CH}_4$

Emissions reduced by 60-70%



## Nutritive modulation of metabolism Dietary supplements



Tannins



Diallyl disulfide



Brown seaweed  
(Phlorotannins)

Improve milk productivity, milk quality and health of milking cows (Nguyen et al., 2022).

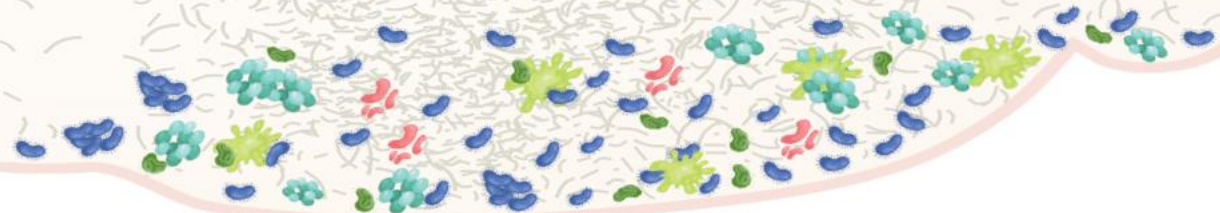
Suppress the population of cellulolytic bacteria (Machado et al., 2014).



Methanogens

$\text{CH}_4$

Emissions reduced by 90%



## Nutritive modulation of metabolism Dietary supplements



Tannins



Diallyl disulfide



Brown seaweed  
(Phlorotannins)



Red seaweed  
(Bromoform)

Improve milk productivity, milk quality and health of milking cows (Nguyen et al., 2022).

Block corrinoid enzymes and inhibit cobamide-dependent methyl group transfer in methanogenesis (Abott, 2020).



Methanogens

$\text{CH}_4$

Emissions reduced by 90%

## Nutritive modulation of metabolism Dietary supplements



Tannins



Diallyl disulfide



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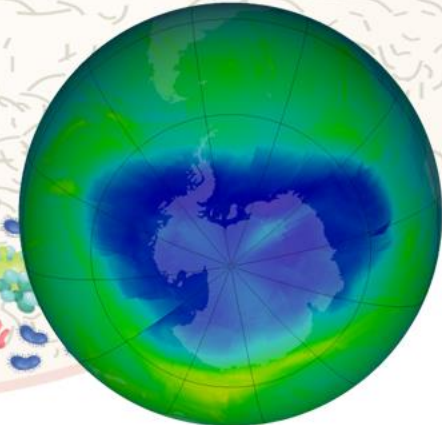


Methanogens

$\text{CH}_4$

Emissions reduced by 90%

However...



## Modulation of metabolism



Tannins



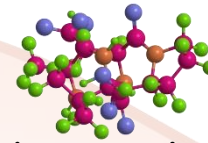
Diallyl disulfide



Brown seaweed  
(Phlorotannins)



Red seaweed  
(bromoform)

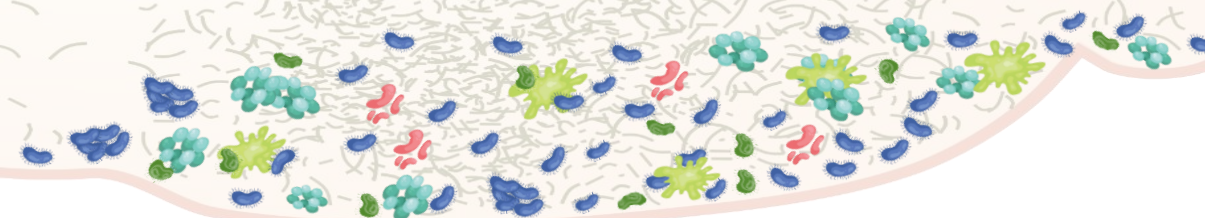


Chemical compounds  
(peptides, bacteriocins, etc.)

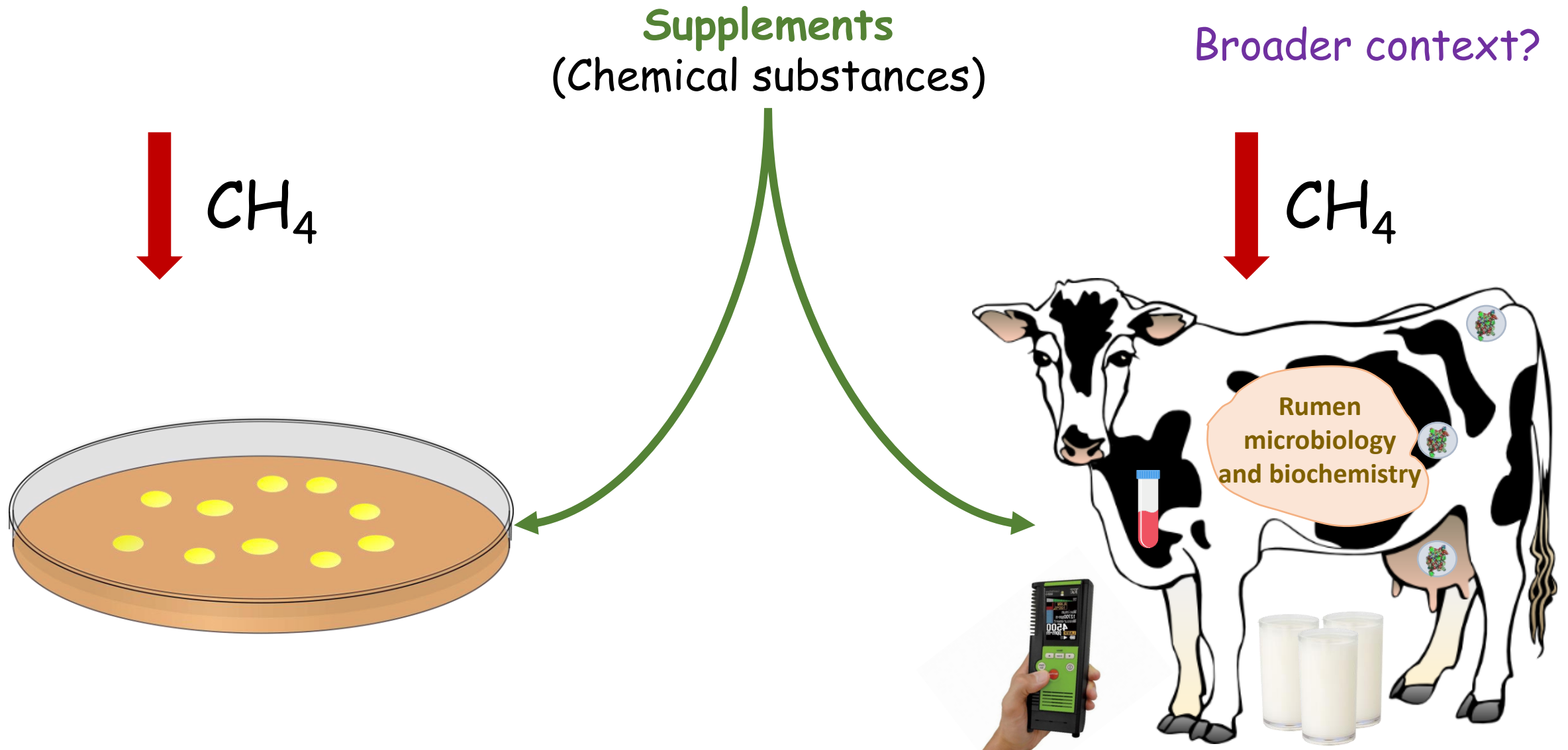
Dramatically reduce methane emissions - *in vitro* studies - questionable?



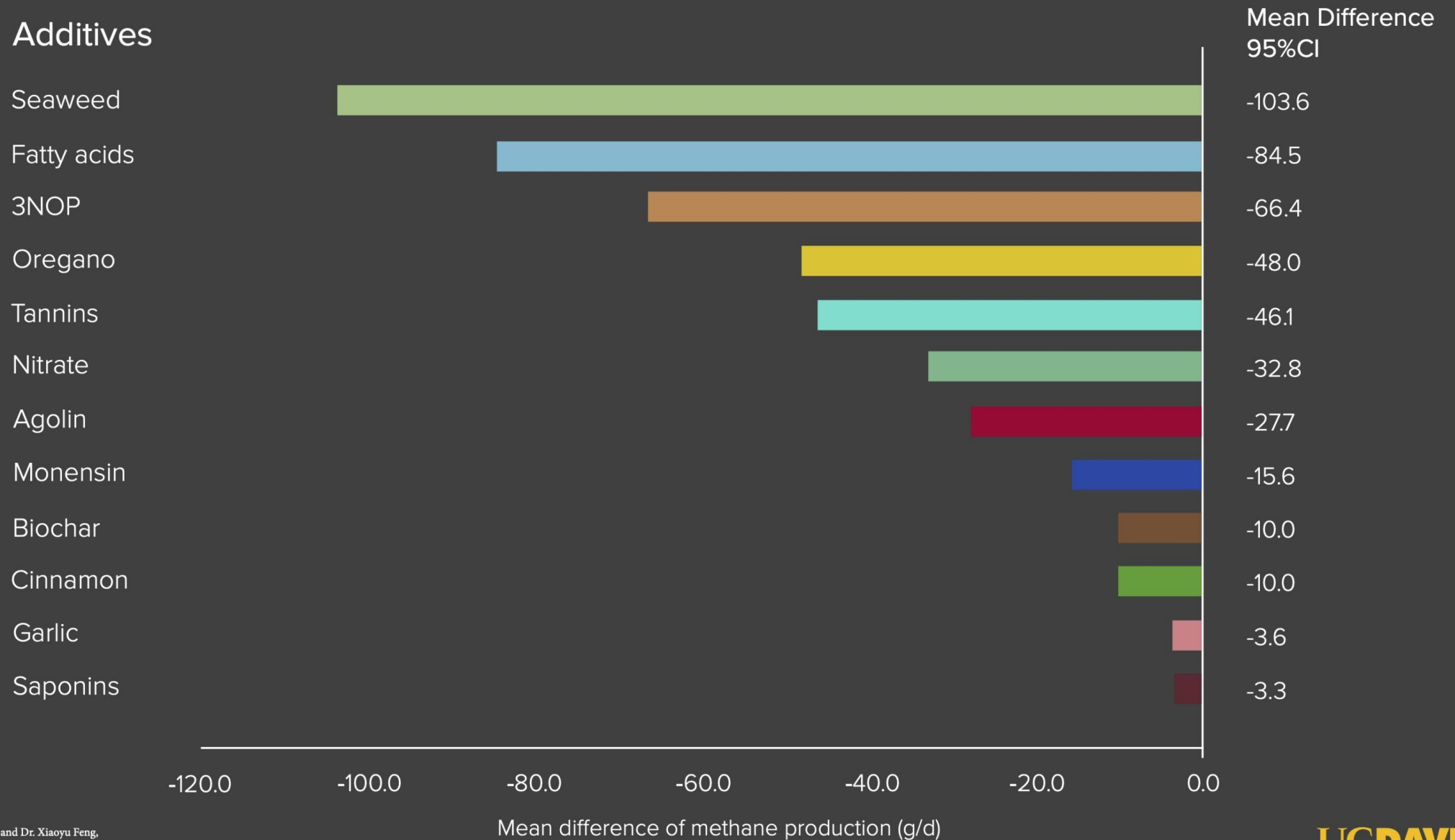
Methanogens



# In Vitro Vs. In Vivo Studies

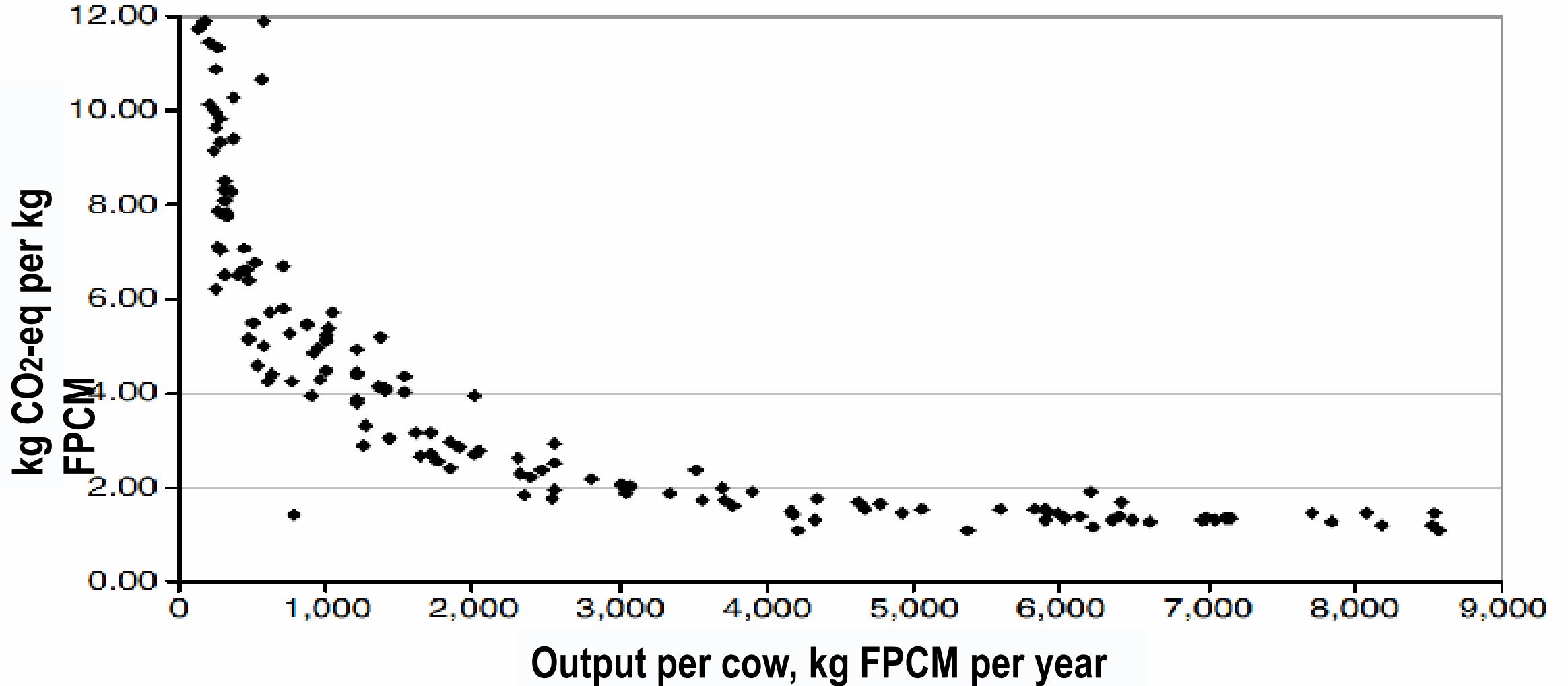


# Methane Reductions from Feed Additives





It is important to consider **the enteric production of CH<sub>4</sub> per unit of livestock product (kg of milk)** because it is necessary to aim for the balance between produced food for a growing human population and GHGs emissions including CH<sub>4</sub>



\*FPCM – fat-protein corrected milk.

# „Carbon footprint“

The total amount of GHG emissions associated with a product, along with its supply-chain. It is usually expressed in kilograms or tones of carbon dioxide equivalent (CO<sub>2</sub>-eq). Methane emitted from enteric fermentation is the major hotspot contributing up to 75 per cent of the total GHG emissions of the dairy sector.

The CF of dairy products from range from: 1.0-6.0 kg CO<sub>2</sub>e per kg fresh dairy products, 4.5-10.0 kg CO<sub>2</sub>e per kg cheese



# „Carbon footprint“

More efficient cow (higher milk production) - lower CF per kg dairy product

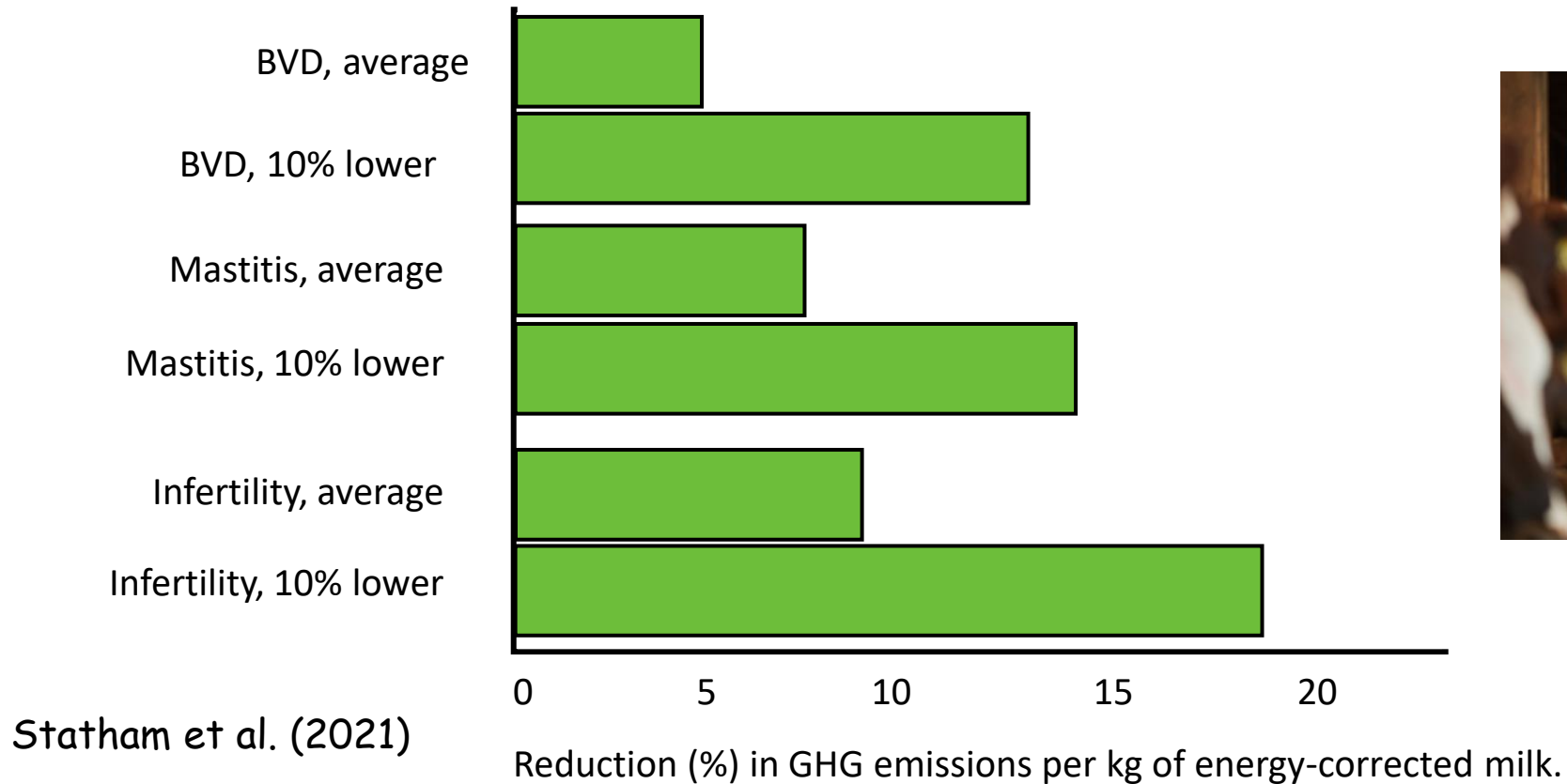


## carbon footprint

The greenhouse gas emissions (carbon footprint) per unit of milk produced has shrunk by more than 63 percent across the U.S. dairy industry since 1944. An additional 25 percent reduction is targeted by 2020.



## GHGs emissions can be significantly reduced by controlling the occurrence of diseases in the herd



**By monitoring health status of dairy cows ALL VETERINARIANS CONTRIBUTE TO ENVIRONMENTAL PROTECTION by decreasing GHGs EMISSION**



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[About the Project](#)

[Project Team](#)

[Activities](#)

[Publications](#)

[Reports](#)

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# Project Team





Science Fund  
of the Republic of Serbia  
Program IDEAS



CH<sub>4</sub>

**Thank you for your attention**

<https://mitimetcattle.vet.bg.ac.rs/services/>



1.1.2022-1.1.2025