OVERVIEW OF THE STATE OF THE ART IN METHANE MITIGATION

David Yañez-Ruiz

Spanish Research Council (CSIC), Granada, Spain
david.yanez@eez.csic.es

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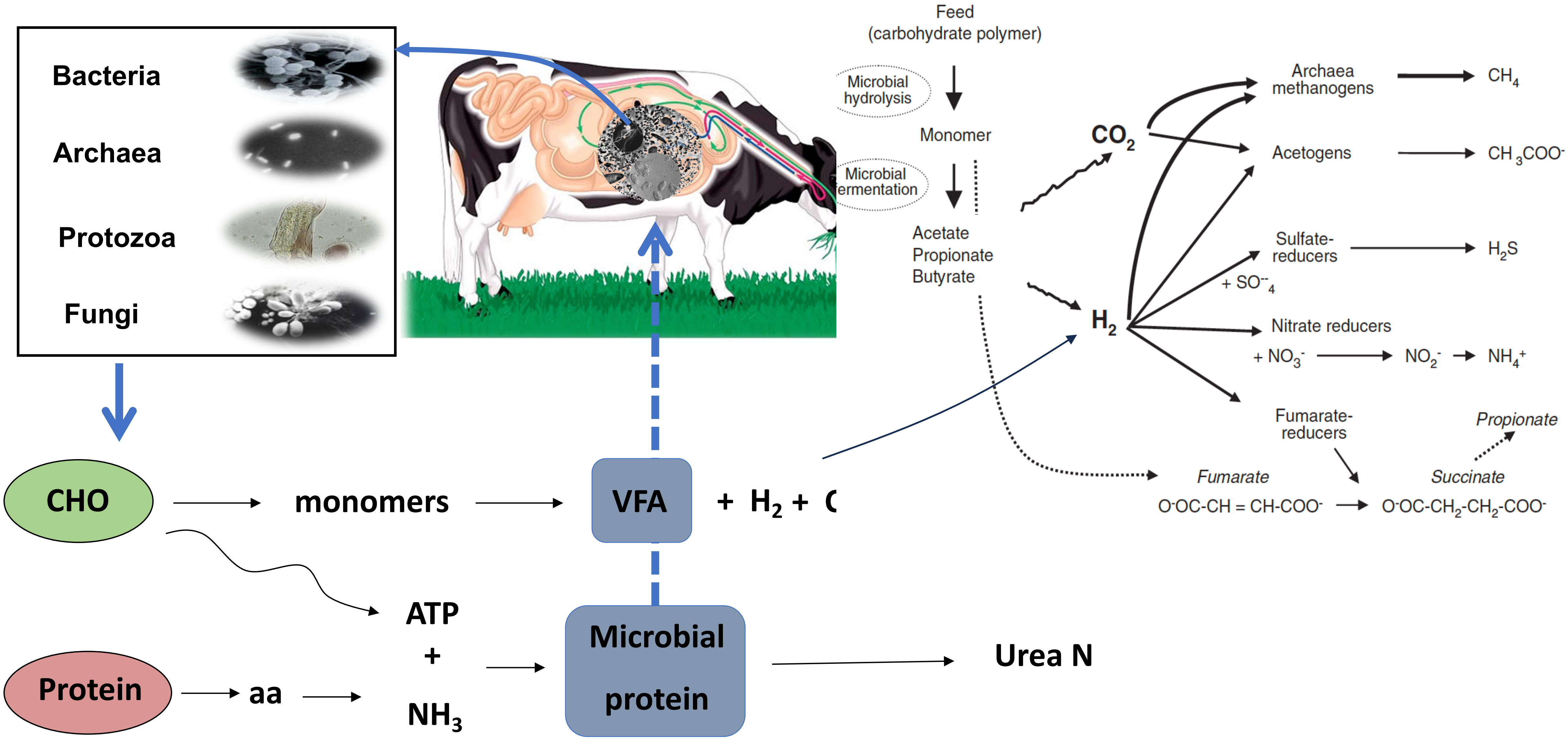
OVERVIEW OF THE STATE OF THE ART IN METHANE MITIGATION

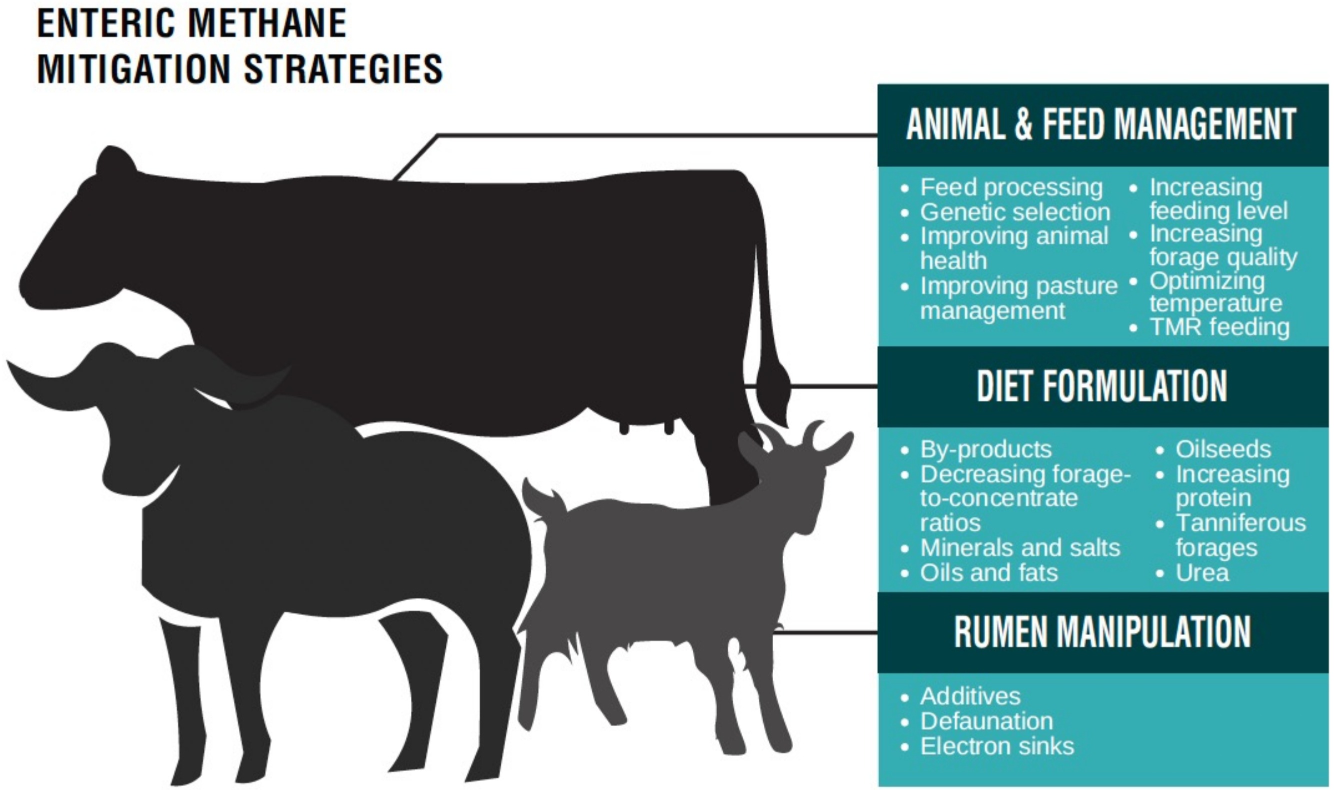
- Microbial production of methane
- Main areas of action
  - Feed additives
  - Vaccines development
  - Animal breeding

Enteric methane production



The rumen microbiome

Methane mitigation strategies



- Feed additives
- Vaccines development
- Animal breeding

PNAS RESEARCH ARTICLE | SUSTAINABILITY SCIENCE OPEN ACCESS

Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050

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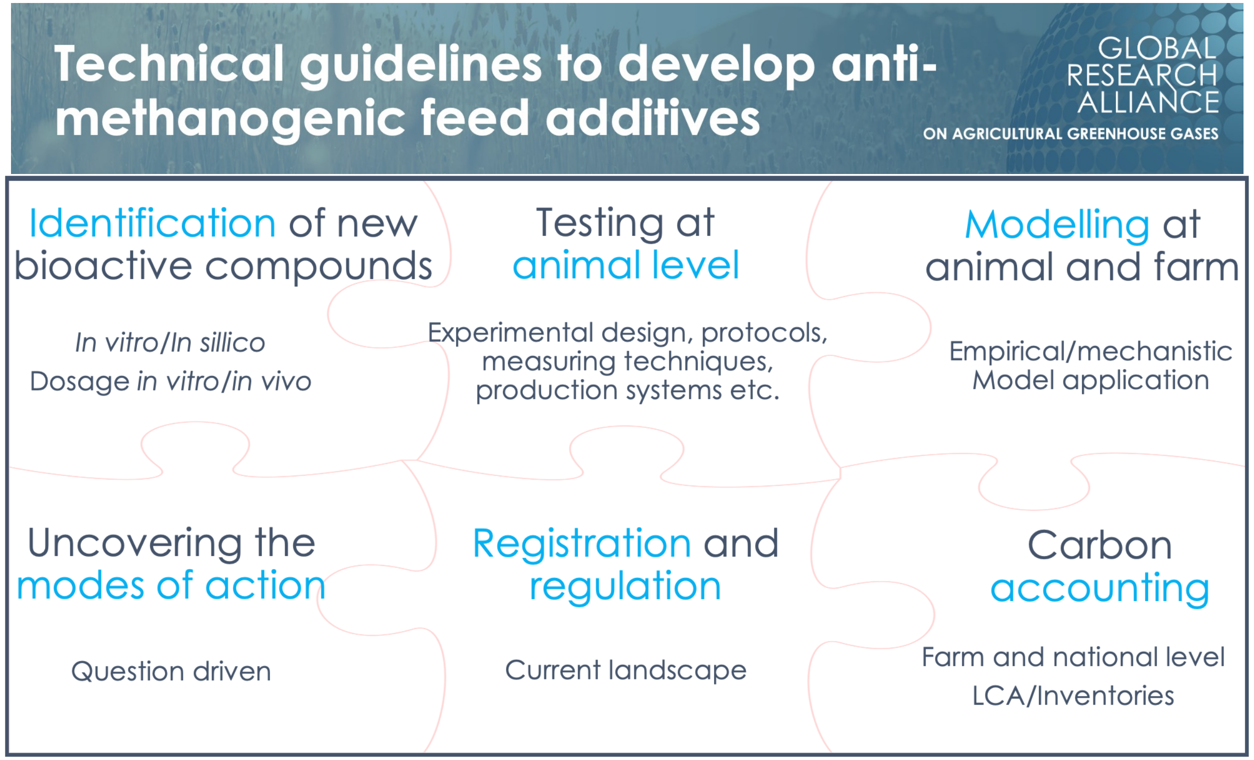
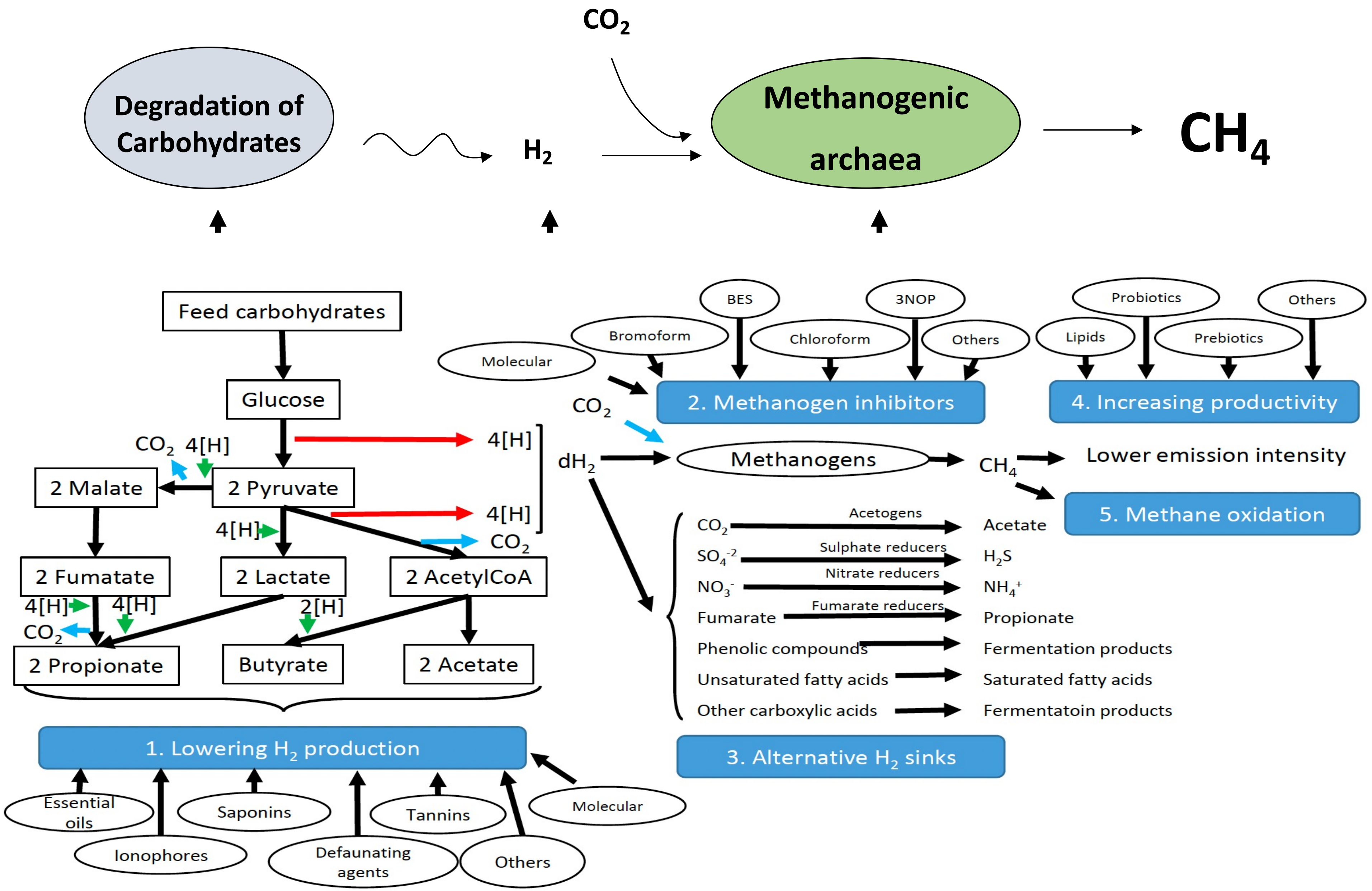
Considerations for a successful CH_4 mitigation strategy

- Efficacy
- Cost
- Regulatory approval
- Adoption / C accounting
- Consumer acceptance
- Longevity – consistency
- Compatible with production system



Journal of Dairy Science®
Official Publication of the American Dairy Science Association®

Feed additives

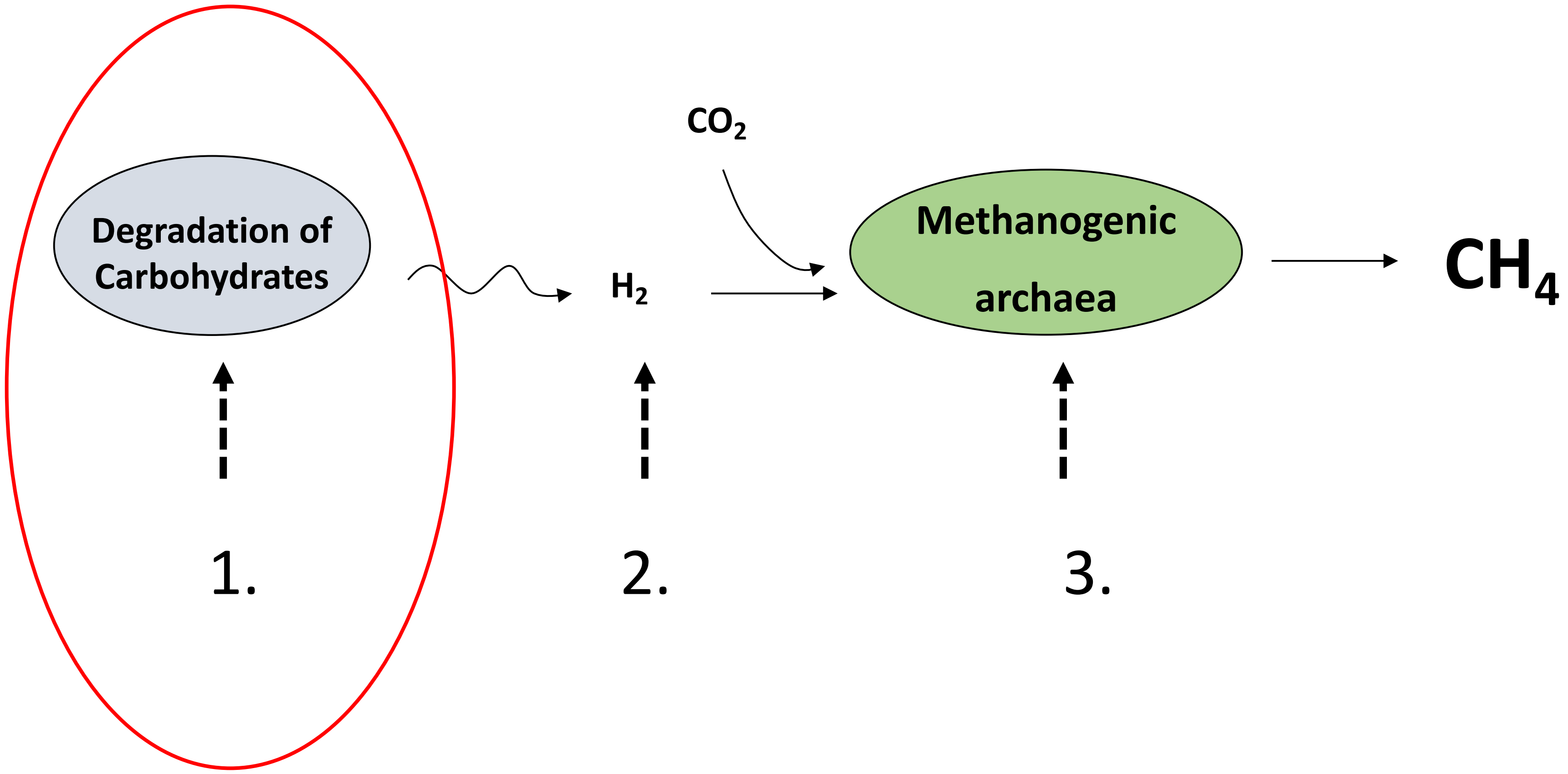


Feed additives

Hegarty *et al.*, 2021

Additive	Efficacy: CH_4 reduction potential[1]	Efficacy: No. of academic papers[2]	Efficacy: Confidence in efficacy[3]
3-Nitrooxypropanol	Very High	> 20	5
Asparagopsis	Very High	< 10	1
Nitrate	High	< 20	4
Essential Oils	Low	< 20	2
Saponin	Low	< 15	1
Tannins	Low	< 15	2
Monensin	Low	> 20	5
Microalgae	Low	< 5	1
Biochar	Low	< 5	1
Bacterial Direct Fed Microbes	Low	< 15	2
Fungal Direct Fed Microbes	Low	< 15	1

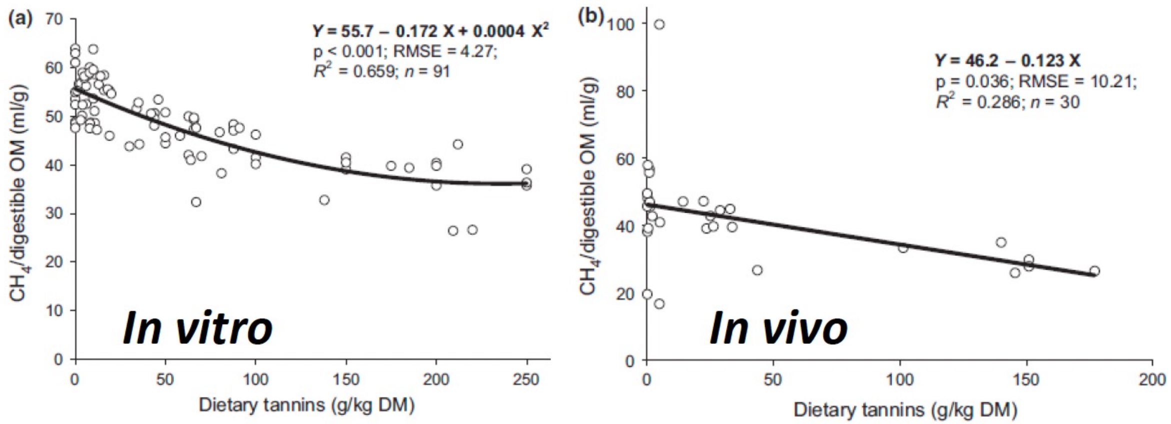
Feed additives

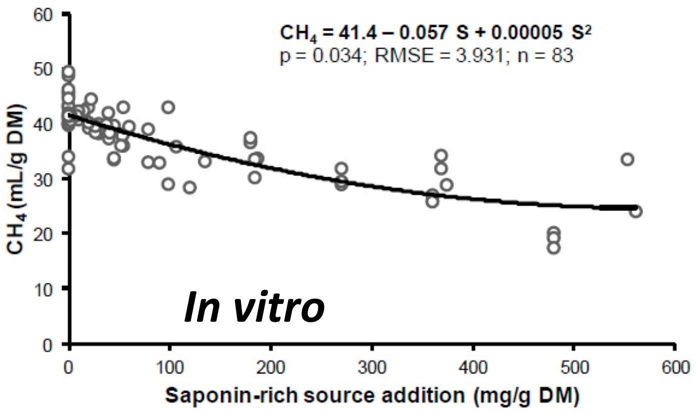


1. Feed additives

Plant secondary compounds:

Condensed/hydrolysable tannins, saponins, essential oils





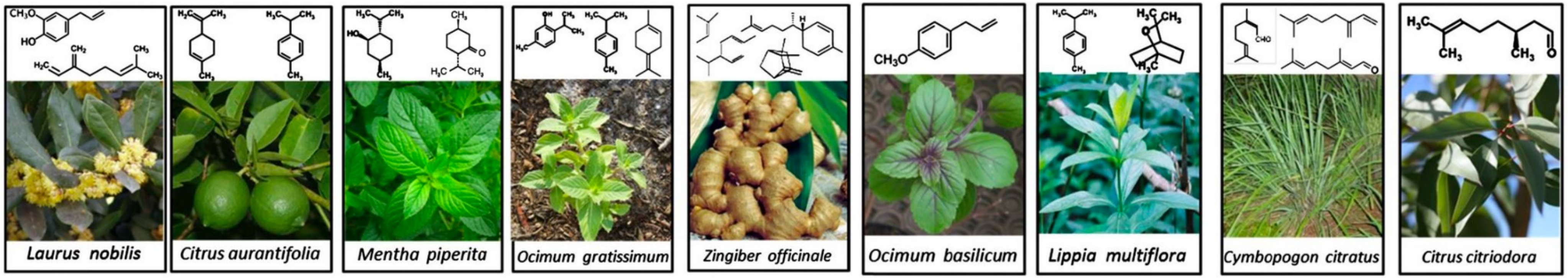
- Inhibit protozoa, some methanogens
- Decrease digestibility and DMI

- Very few in vivo showing reduced CH4

1. Feed additives

Plant secondary compounds:

Condensed/hydrolysable tannins, saponins, essential oils



.....

- Non-specific antimicrobial activity
- Numerous sources and levels tested in vitro for CH_4 effects
- Mainly: thyme, oregano, cinnamon, and garlic or their (thymol, carvacrol, cinnamaldehyde, and allicin)
- Varying responses (some positive)
- Several commercial blends, very few in vivo studies confirm anti methanogenic property

1. Feed additives

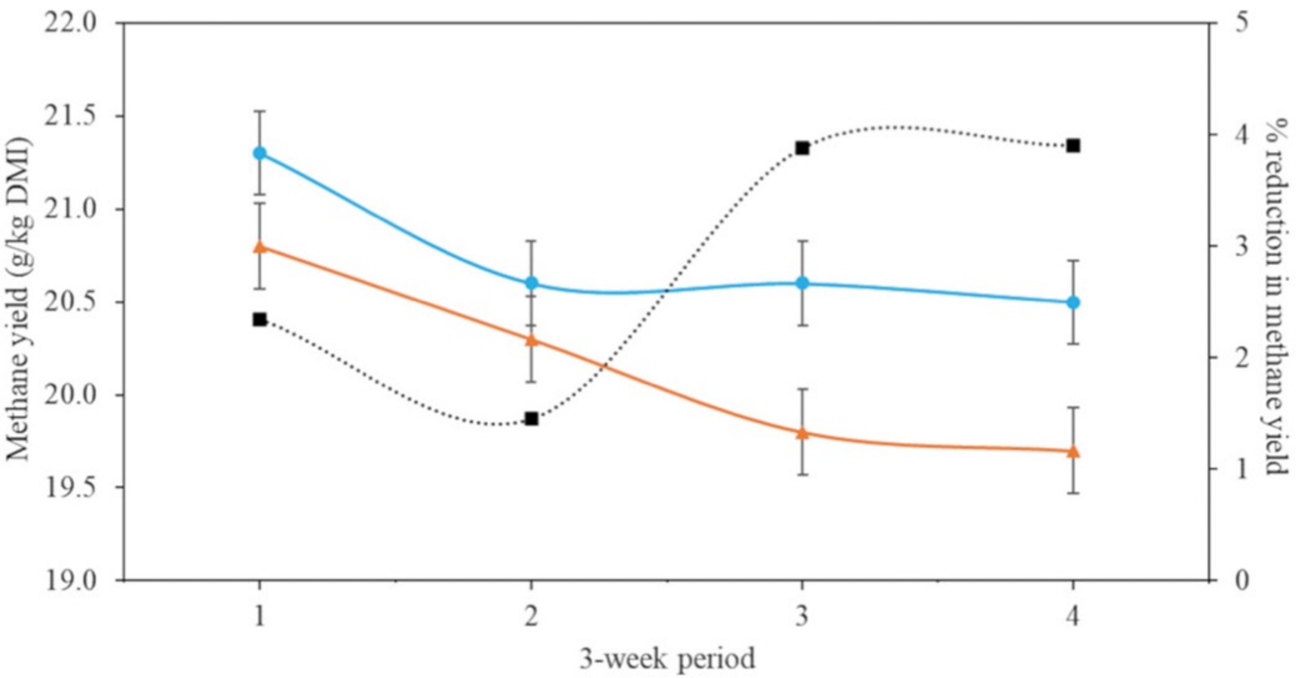
Plant secondary compounds:

Condensed/hydrolysable tannins, saponins, essential oils

J. Dairy Sci. TBC
https://doi.org/10.3168/jds.2023-23406

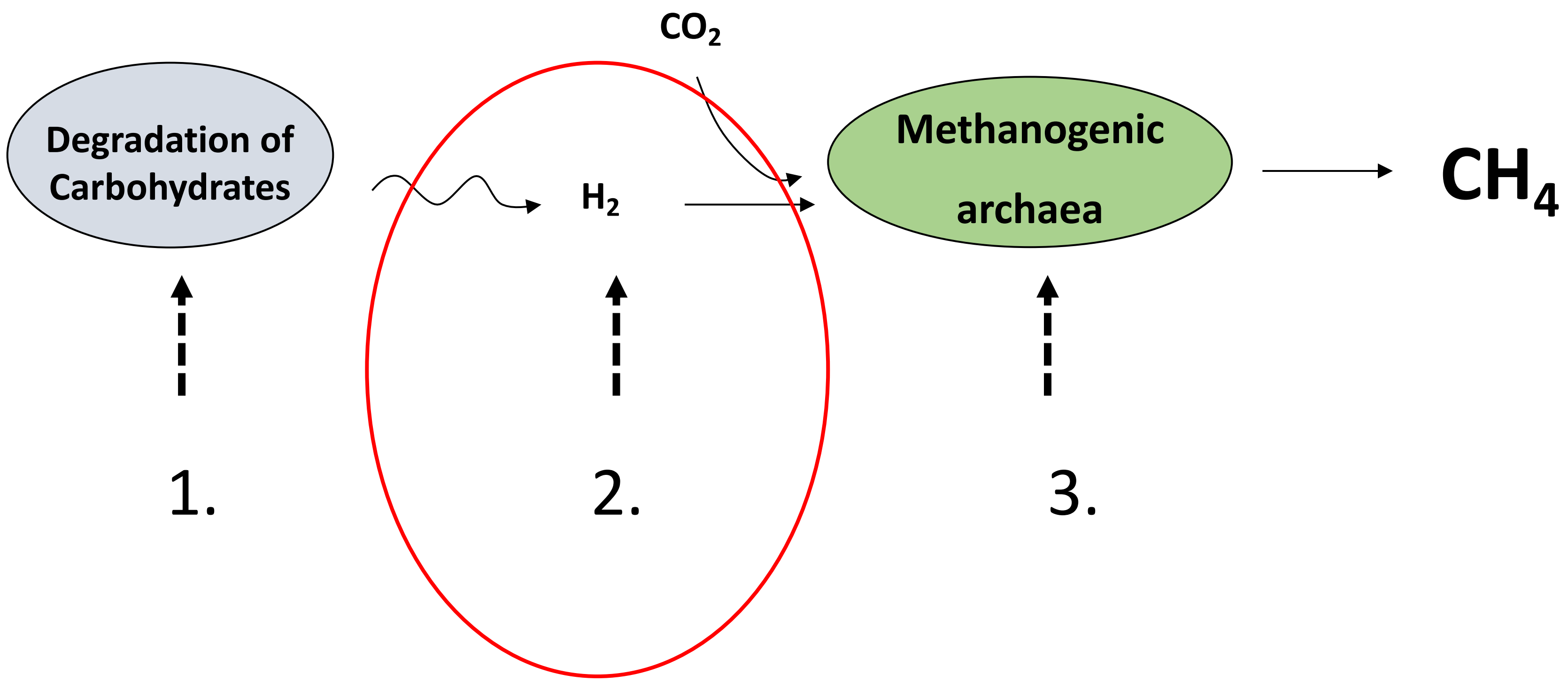
Effect of a blend of cinnamaldehyde, eugenol, and capsicum oleoresin on methane emission and lactation performance of Holstein-Friesian dairy cows

Sanne van Gastelen,[1]* David Yáñez-Ruiz,[2] Hajer Khelil-Arfa,[3] Alexandra Blanchard,[3] and André Bannink[1]

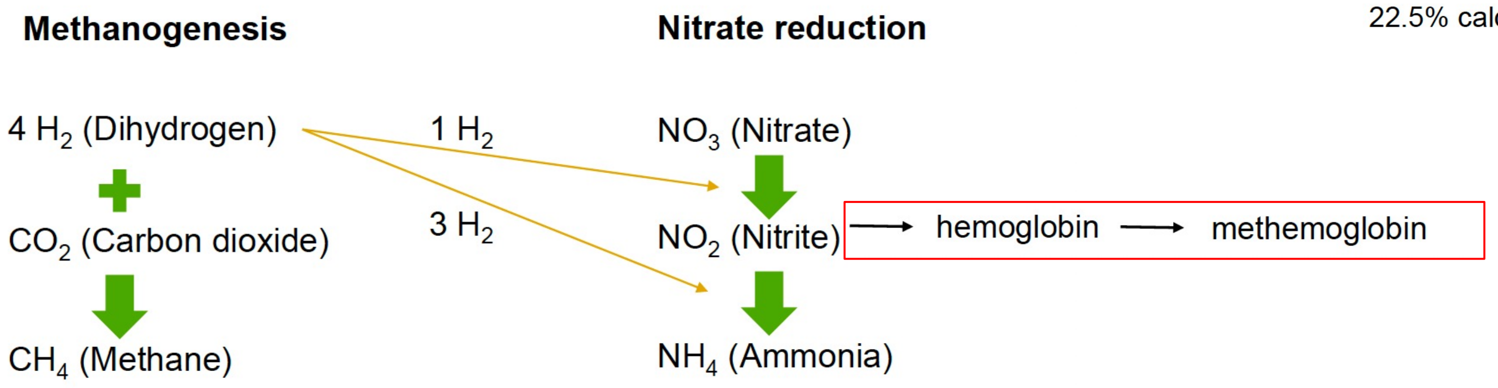


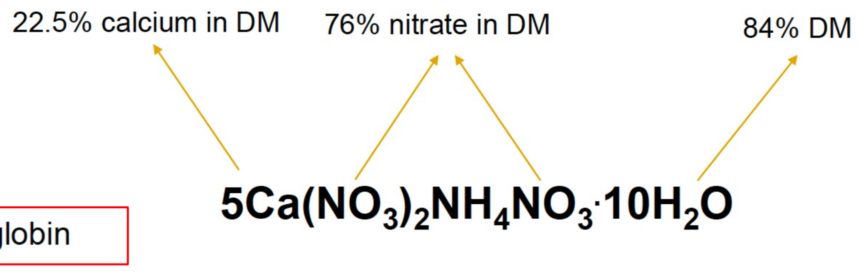
- 2 weeks adaptation
- ↓ H_2
- Modest effect (≈ 5 %)
- No specific registration/approval as CH_4 inhibitor

Feed additives



2. Feed additives: Nitrate

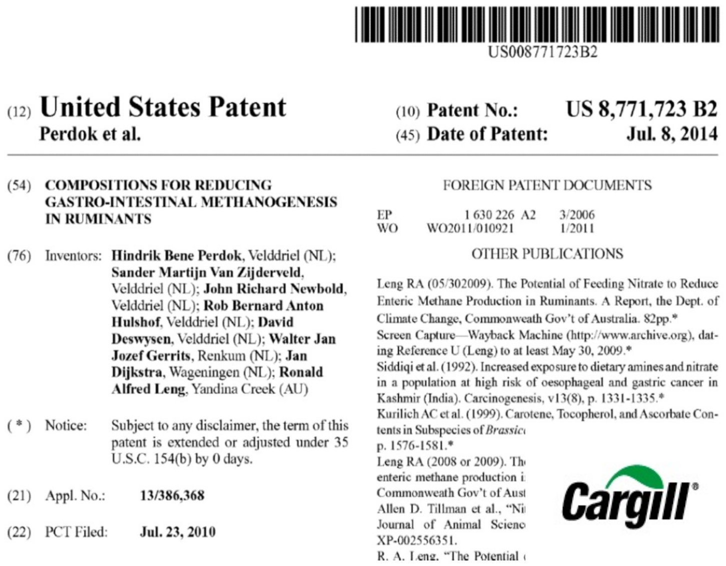




Calciumammoniumnitrate

Feeding 100 g of nitrate should theoretically reduce methane emissions by 25.8 g

- Adaptation neeeded
- Nitrate at a maximum level of 1% of the total diet DM (0.3 g nitrate /kg BW/d)
- 10 % reduction CH4
- Feed ingredient – national regulatory /accounting contexts



US008771723B2

(12) **United States Patent**
Perdok et al.

(10) **Patent No.:** **US 8,771,723 B2**
(45) **Date of Patent:** **Jul. 8, 2014**

(54) **COMPOSITIONS FOR REDUCING GASTRO-INTESTINAL METHANOGENESIS IN RUMINANTS**

(76) Inventors: **Hindrik Bene Perdok**, Velddriel (NL); **Sander Martijn Van Zijderveld**, Velddriel (NL); **John Richard Newbold**, Velddriel (NL); **Rob Bernard Anton Hulshof**, Velddriel (NL); **David Deswysen**, Velddriel (NL); **Walter Jan Jozef Gerrits**, Renkum (NL); **Jan Dijkstra**, Wageningen (NL); **Ronald Alfred Leng**, Yandina Creek (AU)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/386,368**

(22) PCT Filed: **Jul. 23, 2010**

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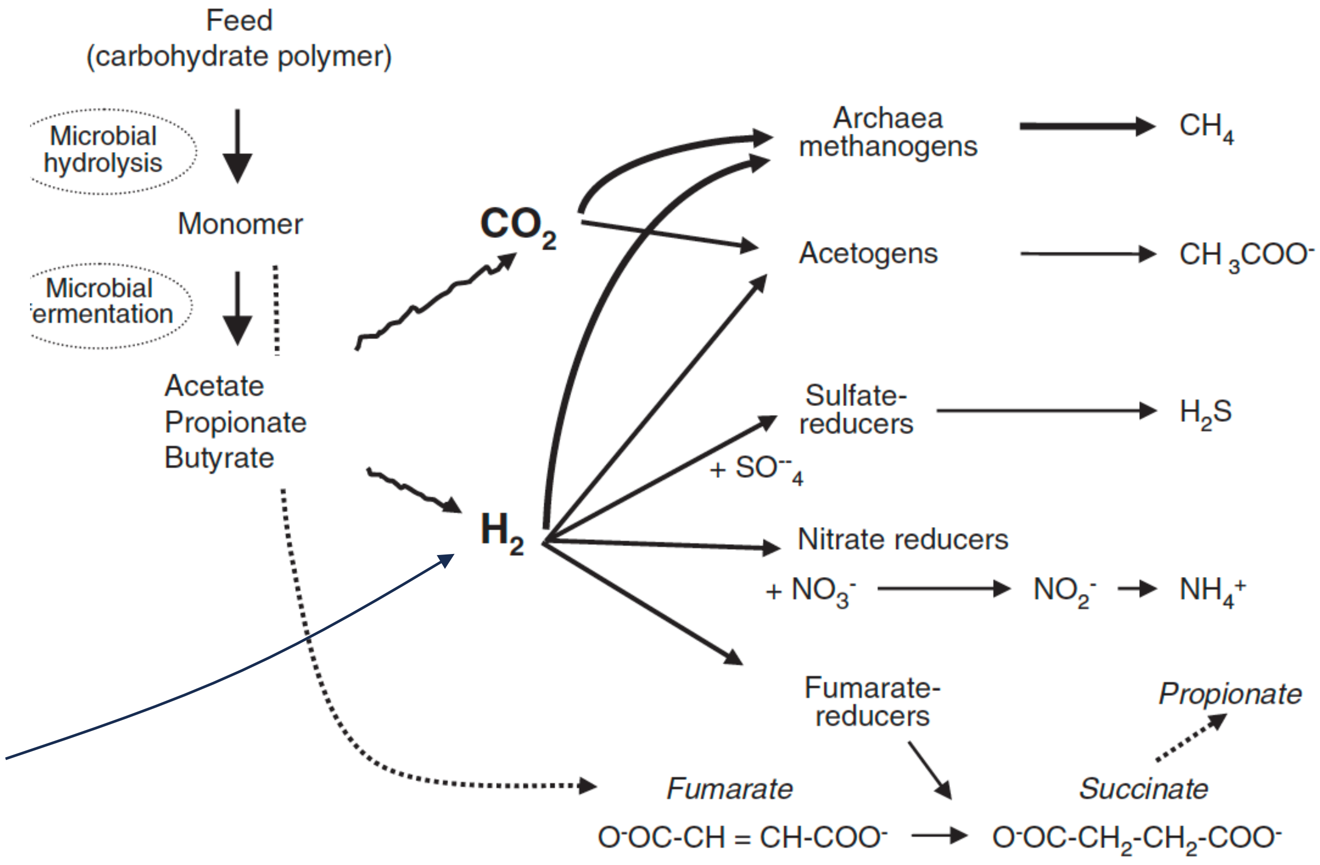
Journal of Animal Scienc
XP-002556351.
R. A. Leng, "The Potential

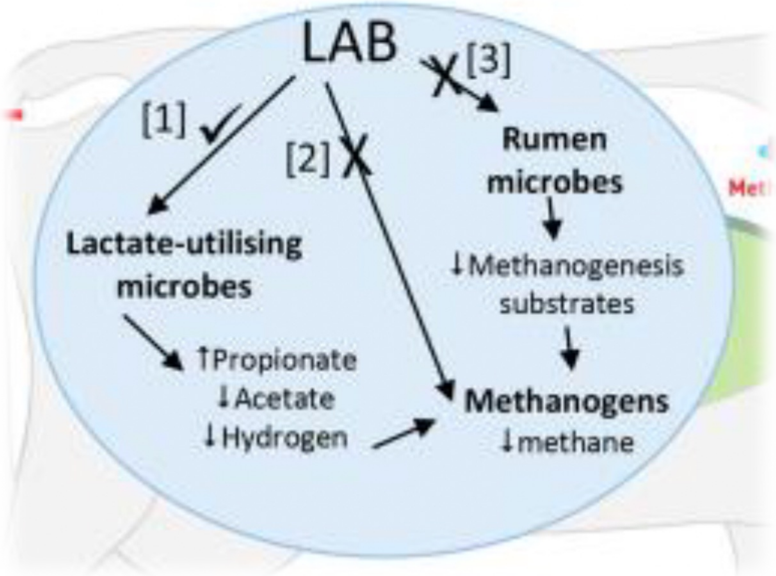
2. Feed additives: probiotics



Acetogens

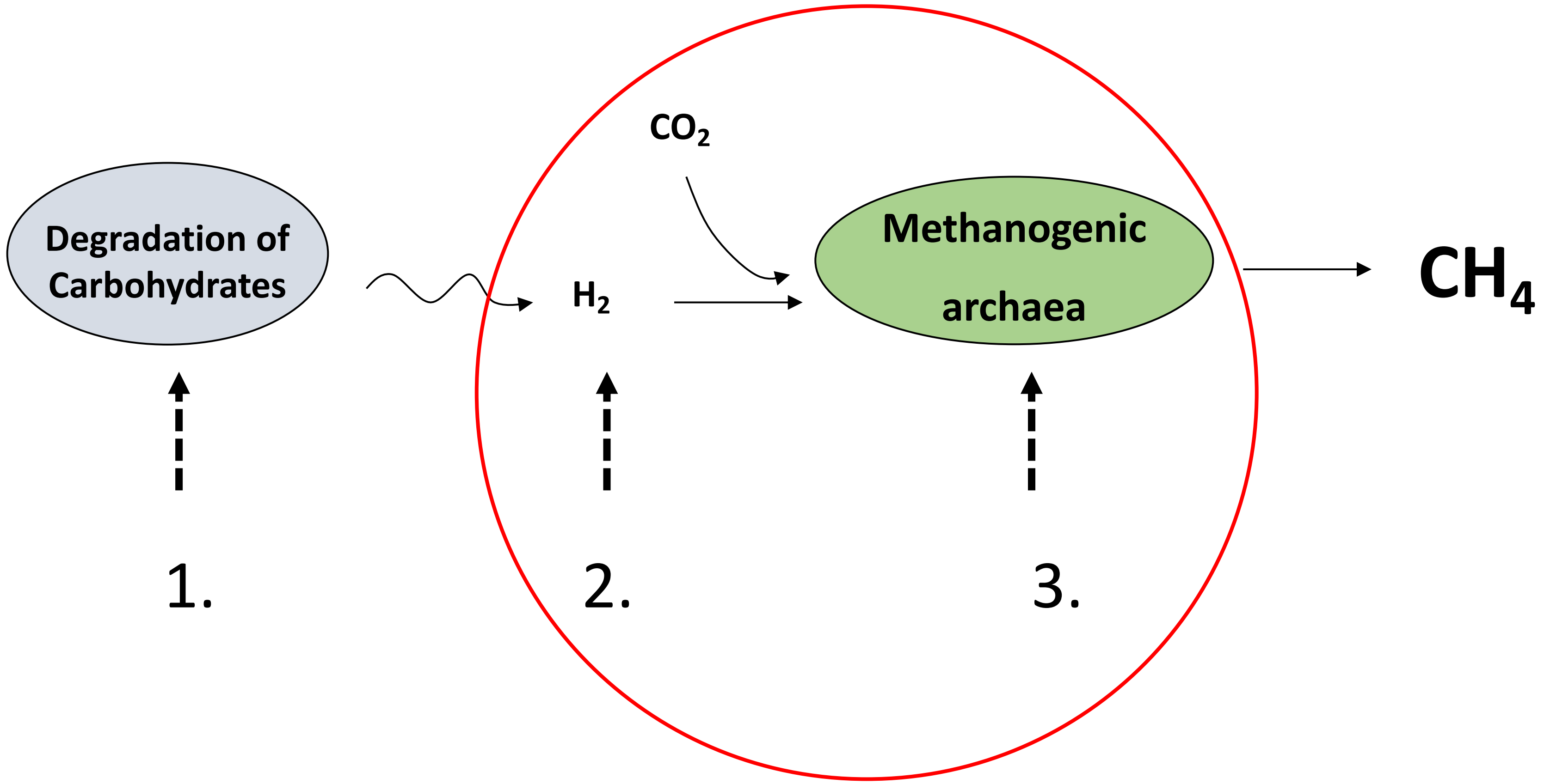
Lactic acid bacteria

LAB



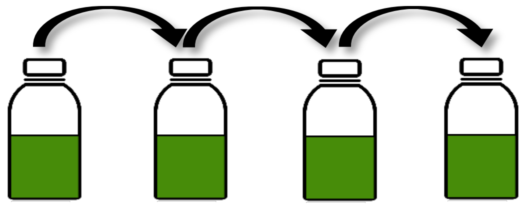
Silage inoculants

Feed additives



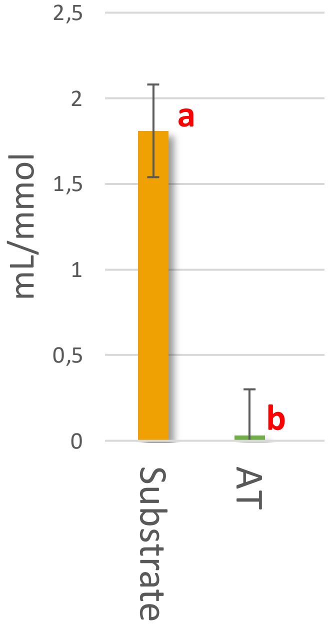
2. Feed additives: H_2 aceptors

Asparagopsis taxiformis **2% DM** + **Phenolic compounds 6 mM**

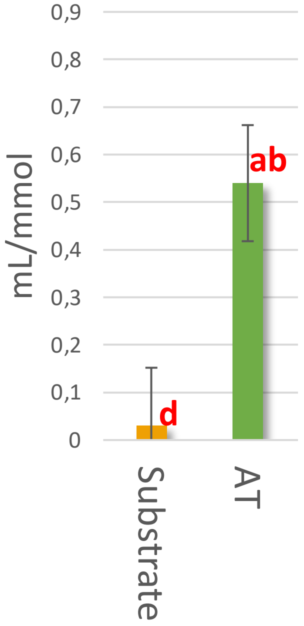


(Romero et al., 2022
Huang et al., 2022)

CH_4



H_2



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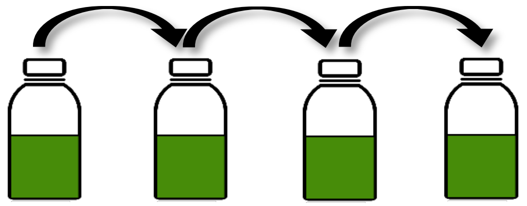
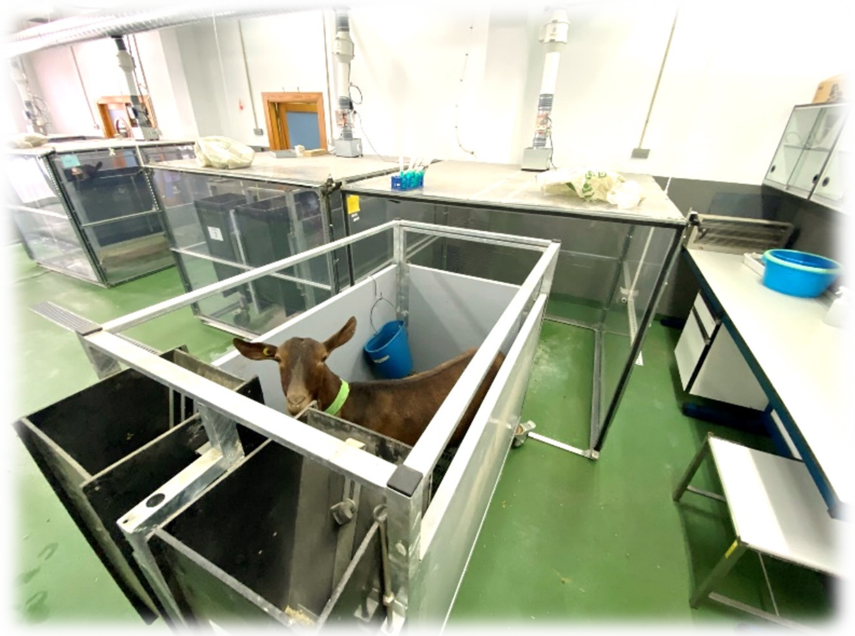
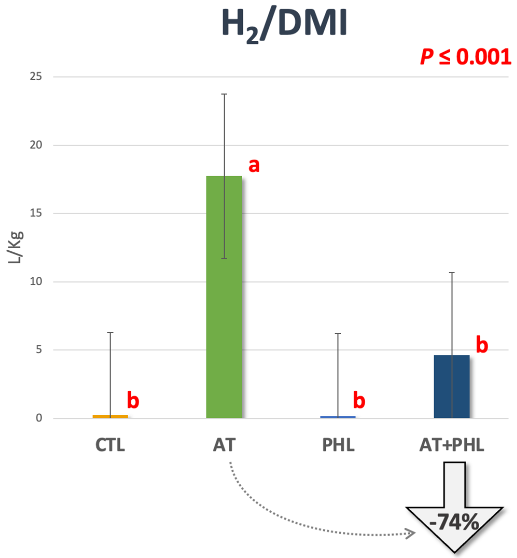
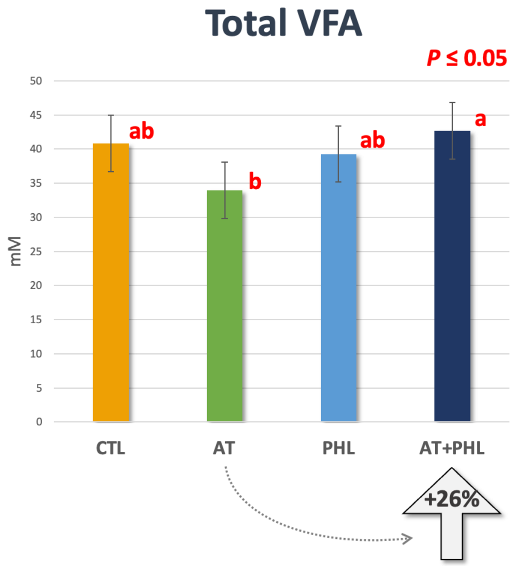


(Romero et al., 2022 Huang et al., 2022)

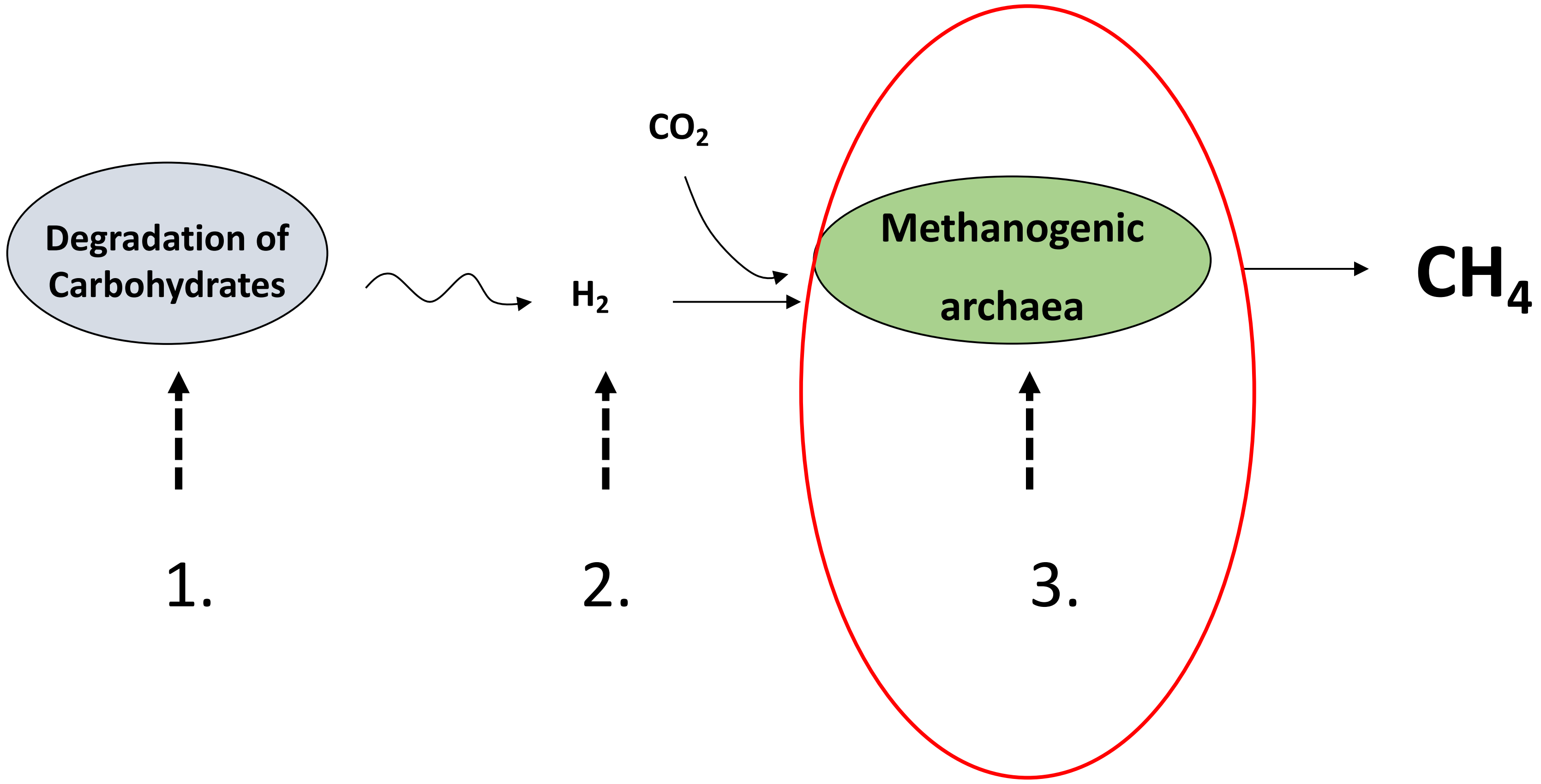






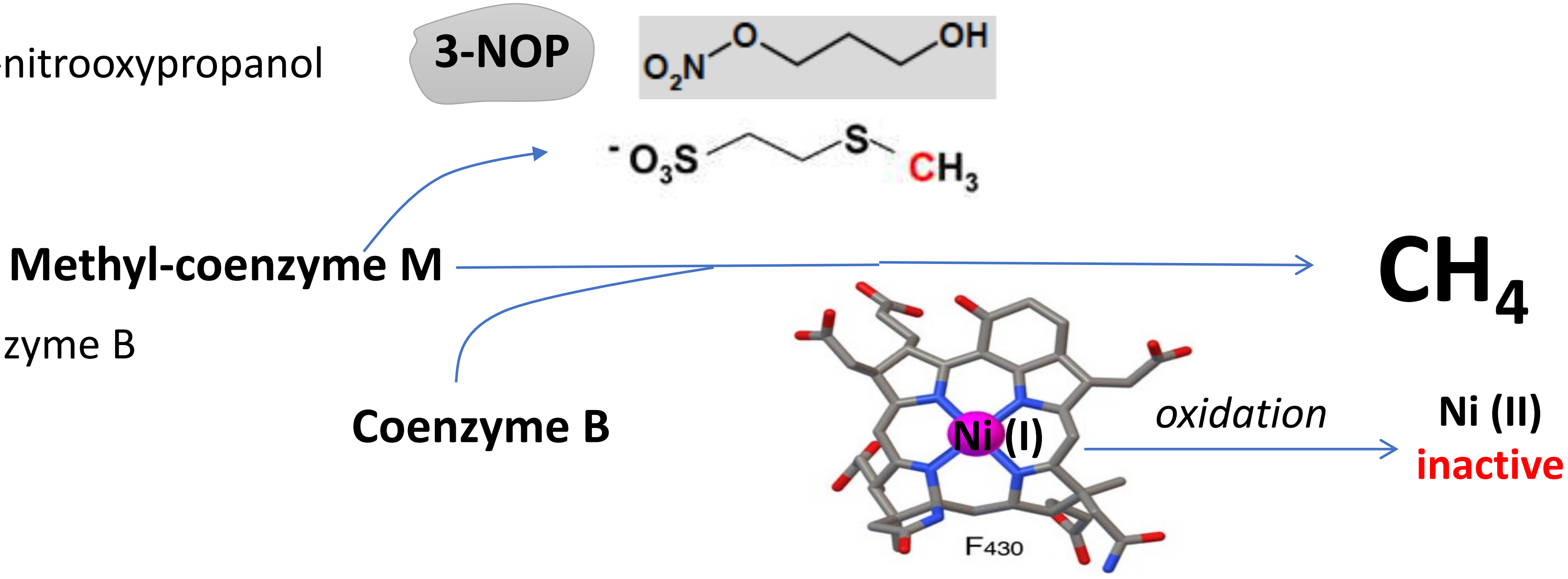
Other combinations??

Feed additives



3. Feed additives: Bovaer®

3-nitrooxypropanol



Coenzyme B

Duin et al., 2016 PNAS

3. Feed additives: Bovaer®

- > 50 studies published
- Dose response. 60 mg/kg DM
- Decreased effectiveness as NDF increases
- Avg. 30% decrease in dairy, 25 - 30% beef backgrounding, 40 - 80% beef feedlot finishing
- Effective in long term studies, no animal production responses
- Current form must be added to the diet (not for grazing animals)

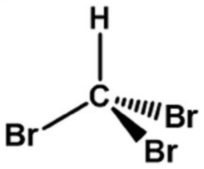

DSM

24-02-2022

DSM receives landmark EU market approval for its methane-reducing feed additive

3. Feed additives: *Asparagopsis*

- Red tropical seaweed (Hawaii, Mediterranean, Australia)
- Can be grown in tanks, dried or extracted
- Blocks the last step of methanogenesis in the rumen
- Bioactive component is bromoform (haloform, similar to chloroform)
  - Animal/human health concerns (bromoform is probable human carcinogen; EPA, 2000)
  - Residues of bromoform have been detected in milk in some (Stefenoni et al., 2021), but not other studies (Li et al., 2016; Kinley et al., 2020; Roque et al., 2021).
- Up to 90% reduction in methane (beef, grain diets), lack of data on animal production
- Emissions associated with producing, harvesting, drying & shipping may offset CH_4 reduction
- Alternative approaches to bromoform production - yeast??



Bromoform
$\Delta H_{C\text{-}Br}$ 278 kJ/mol

3. Feed additives: *Asparagopsis*

Romero *et al.*
Journal of Animal Science and Biotechnology (2023) 14:133
https://doi.org/10.1186/s40104-023-00935-z

Journal of Animal Science and Biotechnology

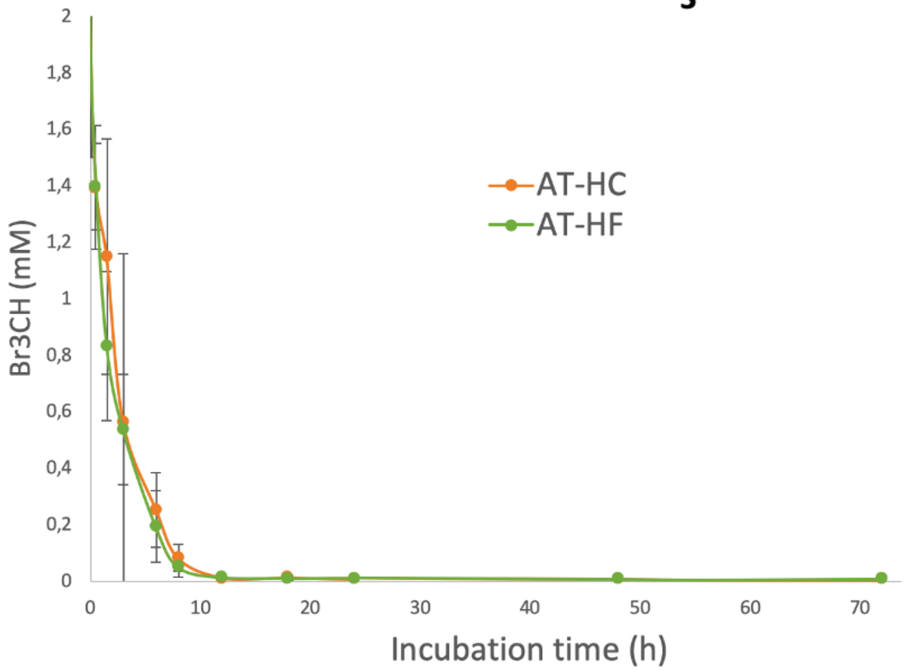
RESEARCH Open Access

Rumen microbial degradation of bromoform from red seaweed (*Asparagopsis taxiformis*) and the impact on rumen fermentation and methanogenic archaea

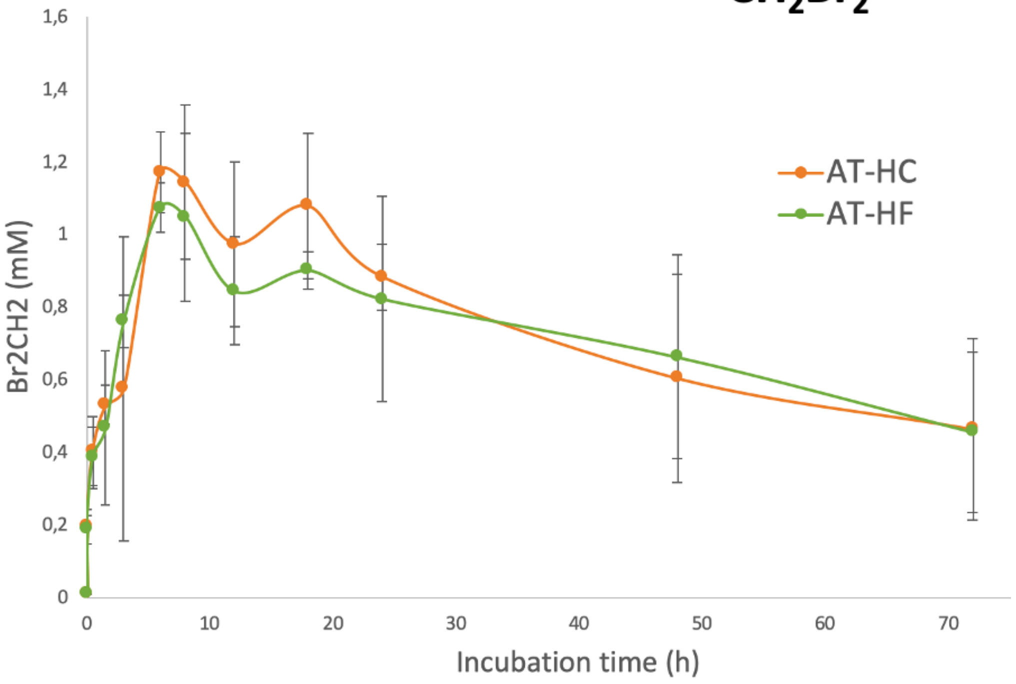
Pedro Romero[1], Alejandro Belanche[2*], Elisabeth Jiménez[1], Rafael Hue:
Joan King Salwen[3], Ermias Kebreab[4] and David R. Yáñez-Ruiz[1*]

Bromoform residues

Bromoform · $CHBr_3$



Dibromomethane - CH_2Br_2



$$CHBr_3 \longrightarrow CH_2Br_2 \longrightarrow CH_3Br \longrightarrow CH_4 + Br^-$$

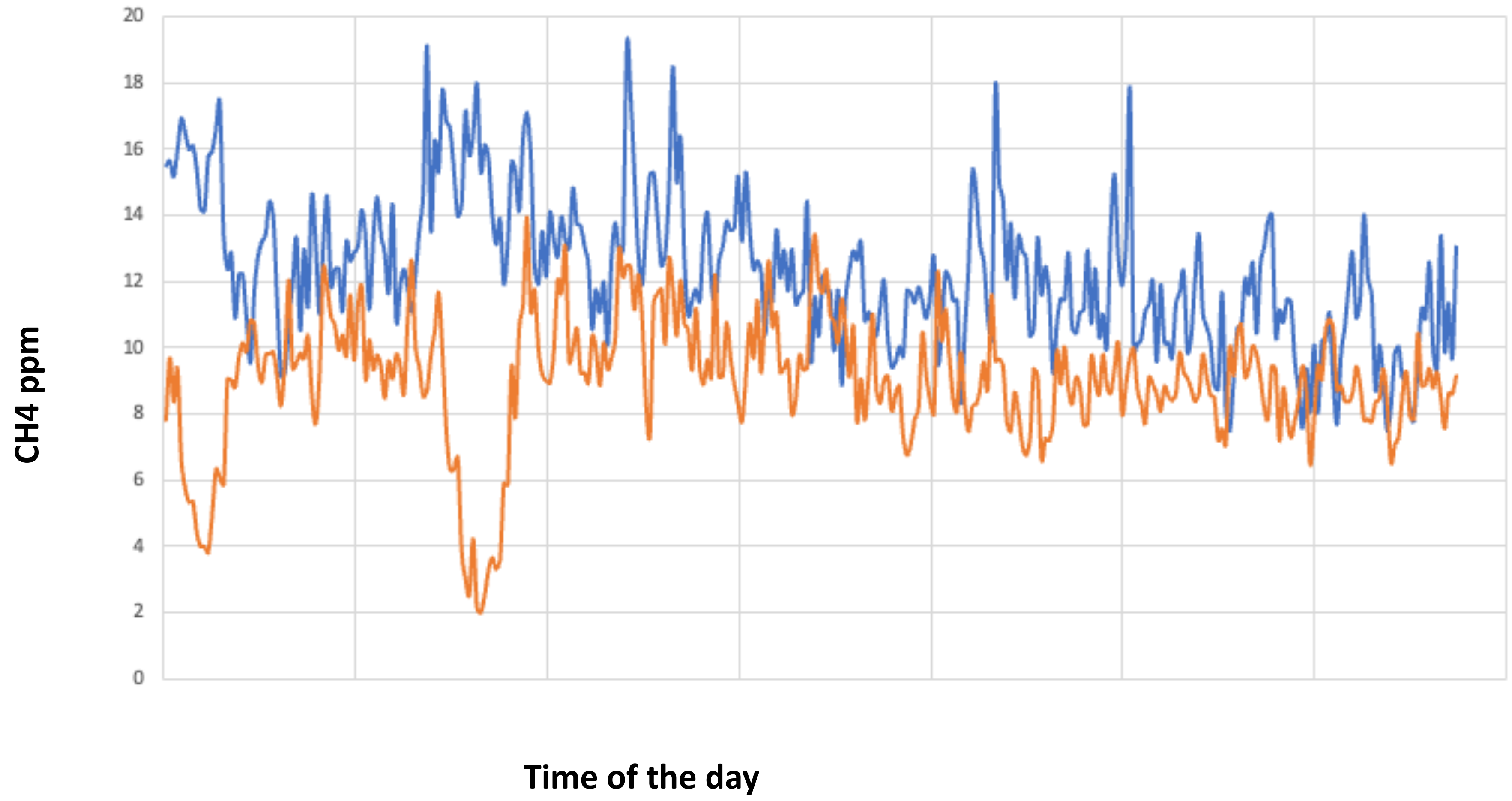
3. Feed additives: *Asparagopsis*

Feed additives

- Longer term studies
- Grazing systems

?

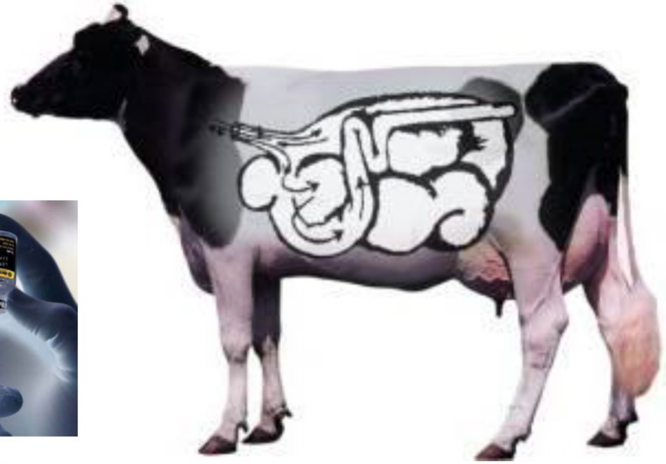
24 hours CH_4 recording

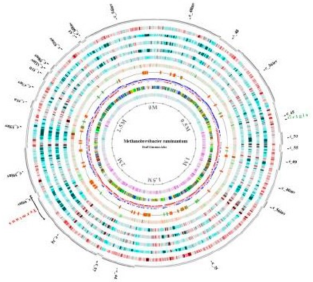


Vaccines development

Development of vaccine: collaboration between Immunology, Genomics & Ecology

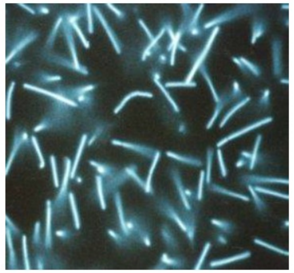
Immunology: Identify methanogen proteins that elicit an immune response (Western blotting). Generate antisera for testing.



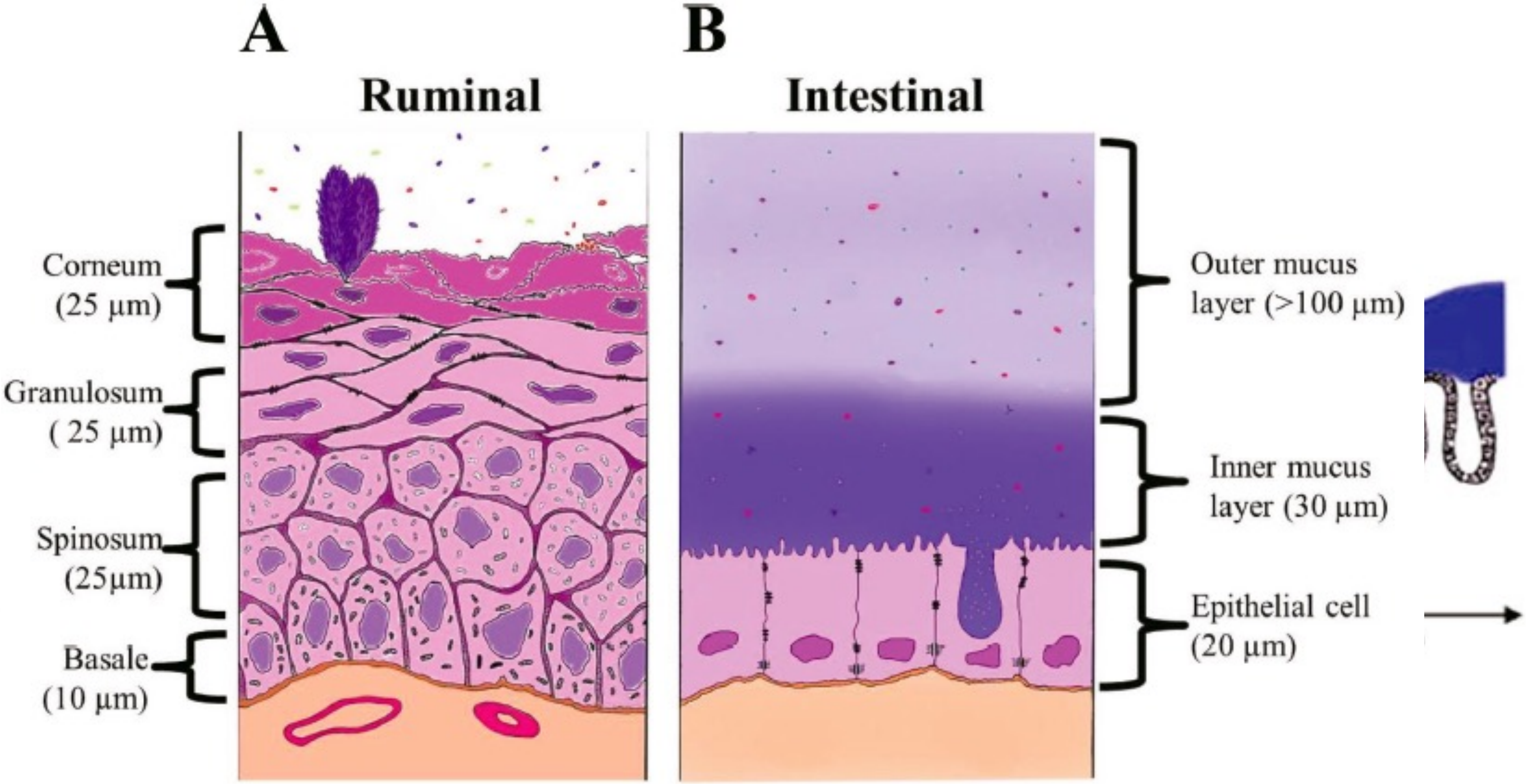


Genomics: Predict methanogen proteins (from analysis of genomic sequences) that could result in immune responses, and are essential for methanogen growth (Bioinformatics)

Rumen ecology: Test impact of anti-methanogen antibodies on methanogens (*in vitro* and *in vivo*)



Vaccines: the rumen specificity



Vaccines: *in vitro* vs. *in vivo*

CH4	Methane Production	Compared Groups	Conditions	References	Booster
In vitro	12.8/14.8% [1] methane reduction in vitro	Sheep vaccinated with methanogen mix vs. prevaccinated/vaccinated with adjuvant or PBS	Primary vaccination with booster 28 days after primary Methane production from rumen liquor incubated for 24 h	[78]	28 days
In vitro	26.26% [1] methane reduction in vitro	Sheep vaccinated with methanogens mix vs. adjuvant and PBS	Primary vaccination with booster 28 days after primary Methane production from rumen liquor incubated for 24 h, corrected for dry-matter intake		
In vivo	Unsuccessful in vivo	Sheep vaccinated with mixes of three or seven methanogens vs. adjuvant and PBS	Primary vaccination Methane production on day 56 or 70 after primary	[79]	
In vivo	12.8% methane reduction in vivo 7.7% methane reduction in vivo, corrected for dry-matter intake	Sheep vaccinated with mix of three methanogens vs. adjuvant and PBS	Primary vaccination with revaccination 153 days after primary Methane production 180–195 days after primary		153 days
In vivo	Unsuccessful in vivo	Sheep vaccinated with mix of seven methanogens vs. adjuvant and PBS			
In vivo	Unsuccessful in vivo	Sheep vaccinated with three methanogens vs. adjuvant	Primary vaccination with booster 42 days after primary Methane production 28 days after vaccination	[80]	
		Sheep vaccinated with three methanogens plus additional methanogens vs. adjuvant			
In vitro	Unsuccessful in vitro	Three semipurified IgY from hens vaccinated with three methanogens vs. semipurified IgY from prevaccinated hens	Primary vaccination with booster on Days 21, 42, 84, and 133 Methane production from rumen liquor incubated for 24 h	[82]	21, 42, 84 and 133 days
In vitro	20% methane increase with anti-*Methanobrevibacter ruminantium* IgY 15% methane increase with anti-*M. smithii* IgY corrected for dry-matter disappearance	Three freeze-dried egg powders from hens vaccinated with three methanogens vs. freeze-dried egg powder from prevaccinated hens	Primary vaccination with booster on Days 21 and 42 Methane production from rumen liquor incubated for 3 h		
In vitro	34% methane reduction with anti-*M. smithii* IgY 52% methane reduction with anti-*Methanosphaera stadtmanae* IgY 66% methane reduction with their combination, corrected for dry-matter disappearance		Primary vaccination with booster on Days 21 and 42 Methane production from rumen liquor incubated for 12 h		21, 42, days
In vitro	Unsuccessful		Primary vaccination with booster on Days 21 and 42 Methane production from rumen liquor incubated for 24 h		
In vitro	49–69% reduction, corrected for dry-matter disappearance	Freeze-dried egg powder from pre-vaccinated hens vs. without egg powder addition	Primary vaccination with booster on Days 21 and 42 Methane production from rumen liquor incubated for 3, 12, and 24 h		28 and 103 days
In vivo		Sheep vaccinated with five methanogens	Primary vaccination with booster		

Baca-González et al., 2020, Vaccines

Animal breeding

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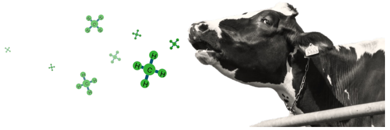

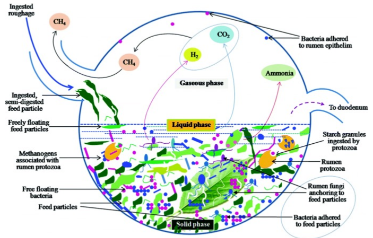

Australia

400 Brahman, composite cattle, Angus (4,250 cattle by 2026)
Microbiome information

Poland

483 Holstein cows

Spain

>3,000 Holstein cows
Microbiome: 439 cows

The Netherlands

8,000 Holstein cows (100 herds: 15,000 cows)
Microbiome: 1,000 cows

Denmark

7,000 Holstein cows

2. In vivo testing: chambers

2. In vivo testing: Greenfeed

Summary

- Many considerations for a succesful development of feed additive

- Three main categories

    - direct archaea inhibitors (> 30 %) – No improvement in productivity
    - combinations ?

- Longer term stiudies

- Grazing systems

- Regulatory/registration constraints

- Vaccine development

Thank you

david.yanez@eez.csic.es

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