75° EAAP Annual Meeting – Florence, 1-5 September 2024
Session 47 – Nutrition management to reduce methane emission and environmental impact, Part 2

Effects of hydrolysable tannin metabolites on rumen microbiota and enteric methane production

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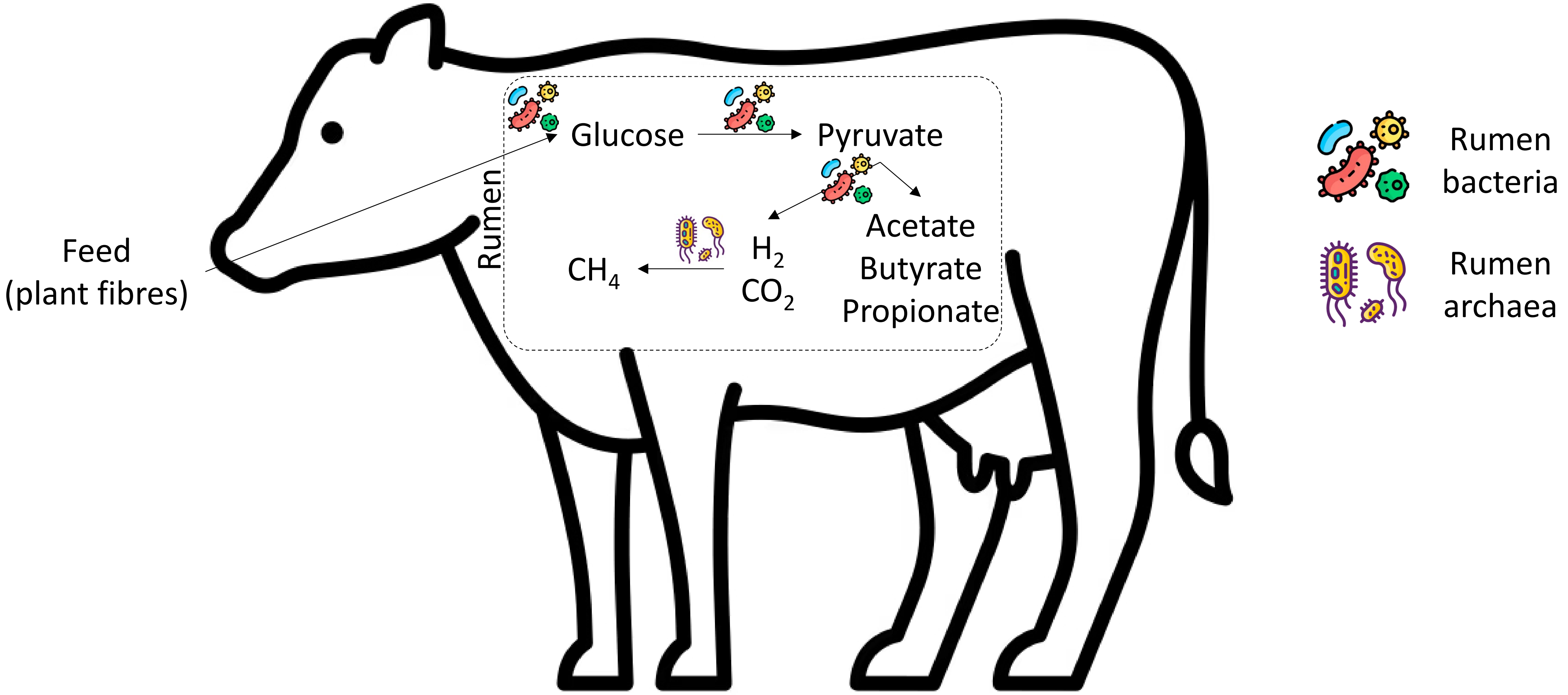
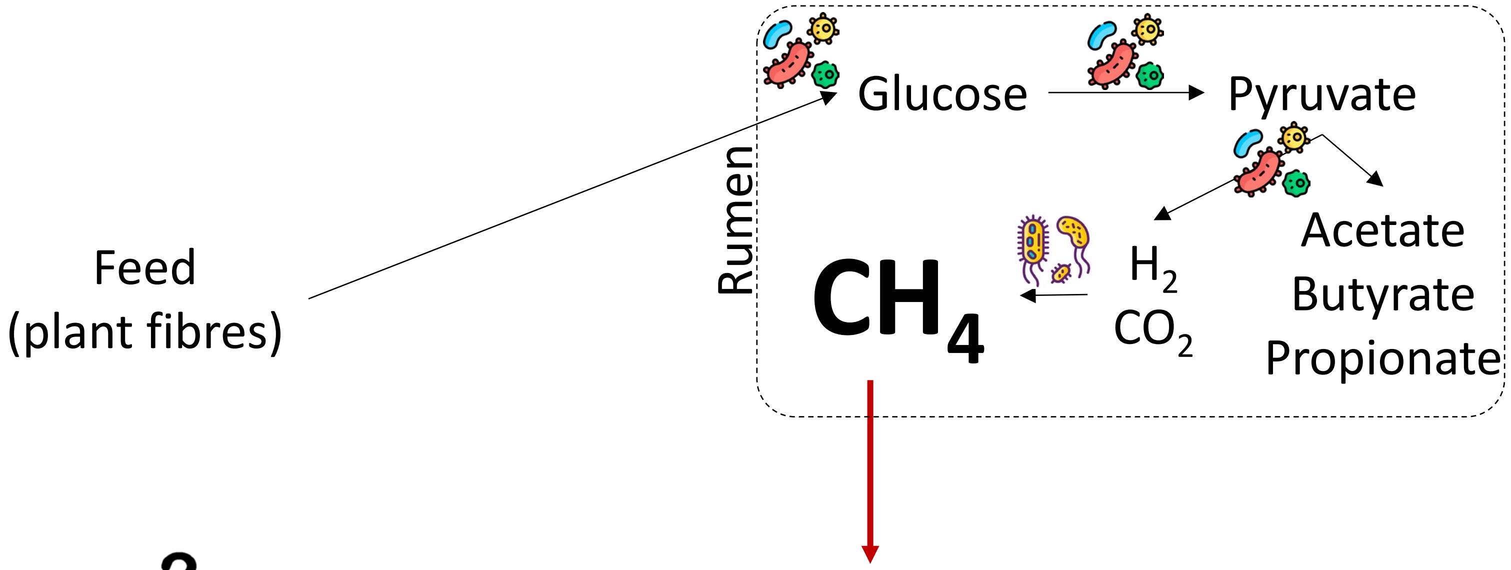
Rumen fermentation



Methane



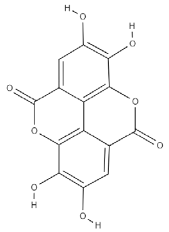
- Higher global warming potential than CO_2
- Methane from enteric fermentation:
  - 45% of livestock related total GHG emissions
  - 90% of livestock related methane emissions

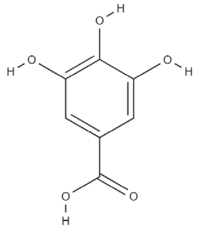
How to reduce ruminant methane emissions?

Tannins

- Plant secondary metabolites
- Bind to macromolecules (proteins, fibres) → reduce feed degradation in rumen

- Classified as:
  - Condensed tannins
  - **Hydrolysable tannins**





Ellagic acid (EA) and gallic acid (GA)

Contents lists available at ScienceDirect

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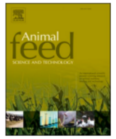


Effect of ellagic and gallic acid on the mitigation of methane production and ammonia formation in an *in vitro* model of short-term rumen fermentation

Michele Manoni[a,*], Melissa Terranova[b], Sergej Amelchanka[b], Luciano Pinotti[a,c], Paolo Silacci[d], Marco Tretola[a,d]

Hohenheim gas test

EA15
EA+GA

- Decreased CH_4 (-22%)
- Decreased ammonia (-17%)

- Lower SCFA (-10%)
- Lower nutrient degradation (-10%)

How EA and GA affect rumen microbial community?

Which microorganisms correlate with methane emissions?

Can rumen microbial modulation reduce methane emissions?

Aim of the study

10-d Rusitec trial to evaluate the effect of EA and GA on rumen microbiota and fermentation

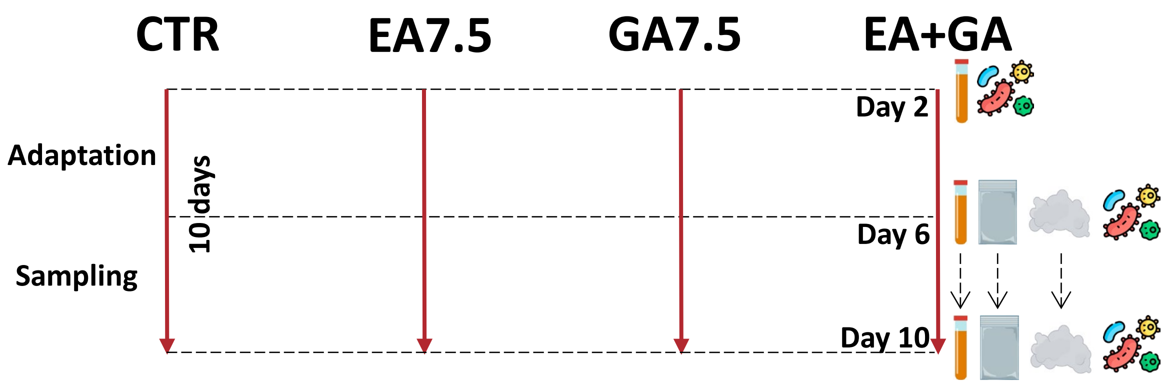
Rumen microbiota
↓
Bacteria and archaea

Correlation between rumen microbiota and methane production

Fermentation
↓
Enteric methane

Methods

- Fresh rumen fluid collection
- Rumen fluid + artificial saliva (buffer)
- Feed substrate (hay + concentrate)



Rumen fluid parameters

Nutrient degradation

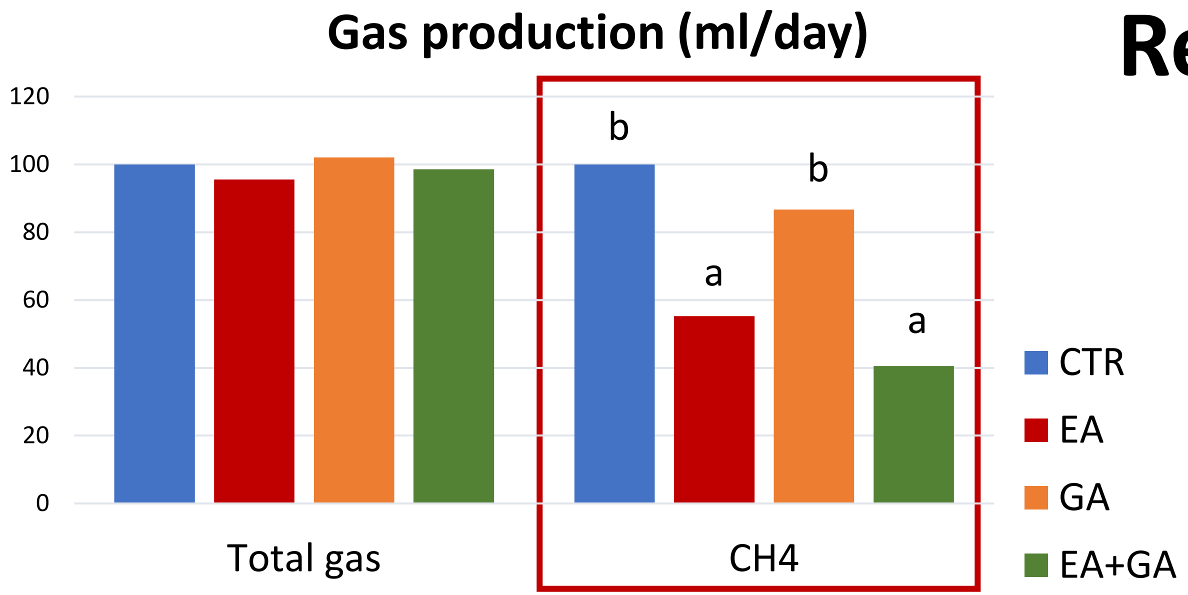
Gas production

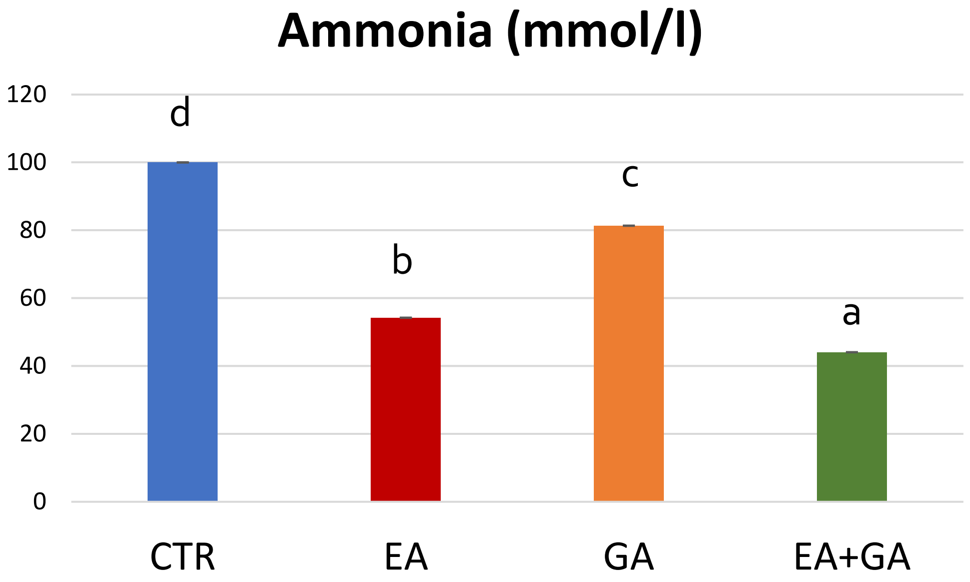
High-throughput sequencing → Raw sequences grouped into **amplicon sequence variants (ASVs)**

Main features:
- 3 donor animals
- 8 experimental units (fermenters)
- 3 runs
- 10 days of experiment

Treatments:
- Control → CTR, 10 g DM
- EA → CTR + EA 7.5 % DM
- GA → CTR + GA 7.5% DM
- EA+GA → CTR + EA + GA, both 7.5 % DM

Results

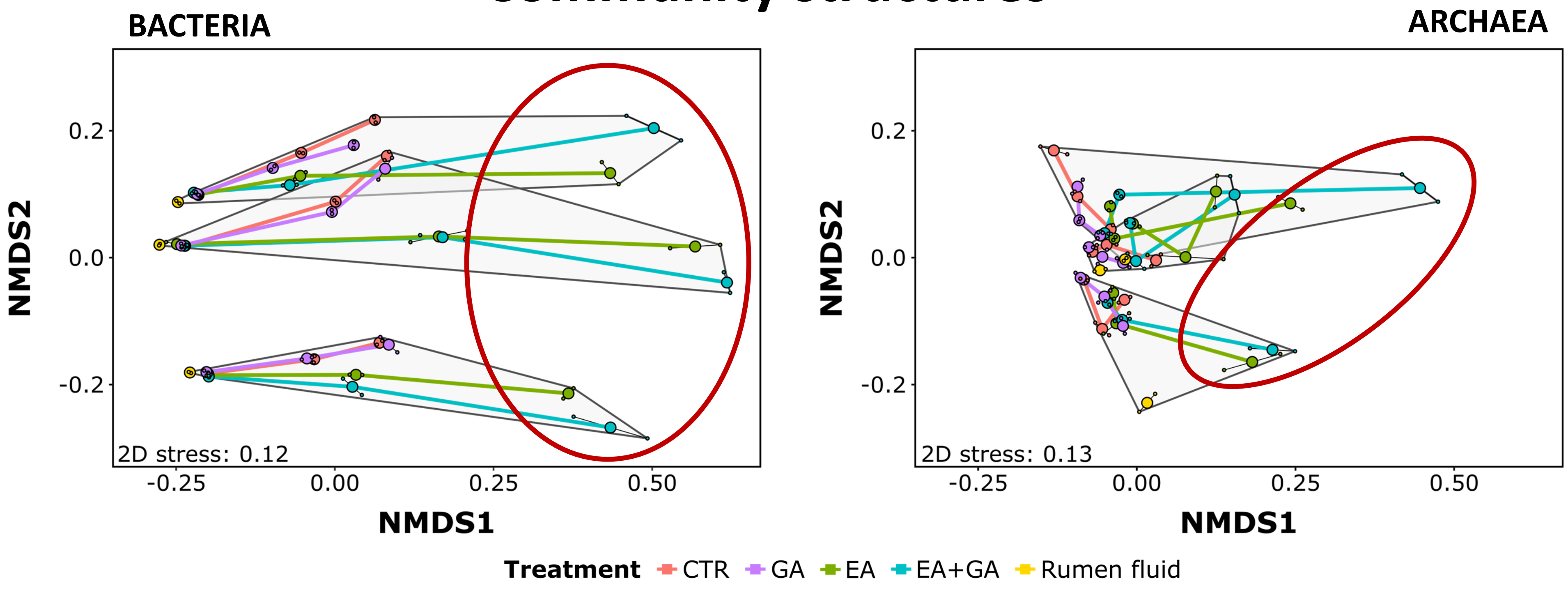




- Total gas production was not altered
- EA and EA+GA reduced CH_4 production (-53%)
- EA and EA+GA reduced total SCFA (-21%) and nutrient degradation
- EA and EA+GA reduced ammonia formation (-51%)

Results

Community structures

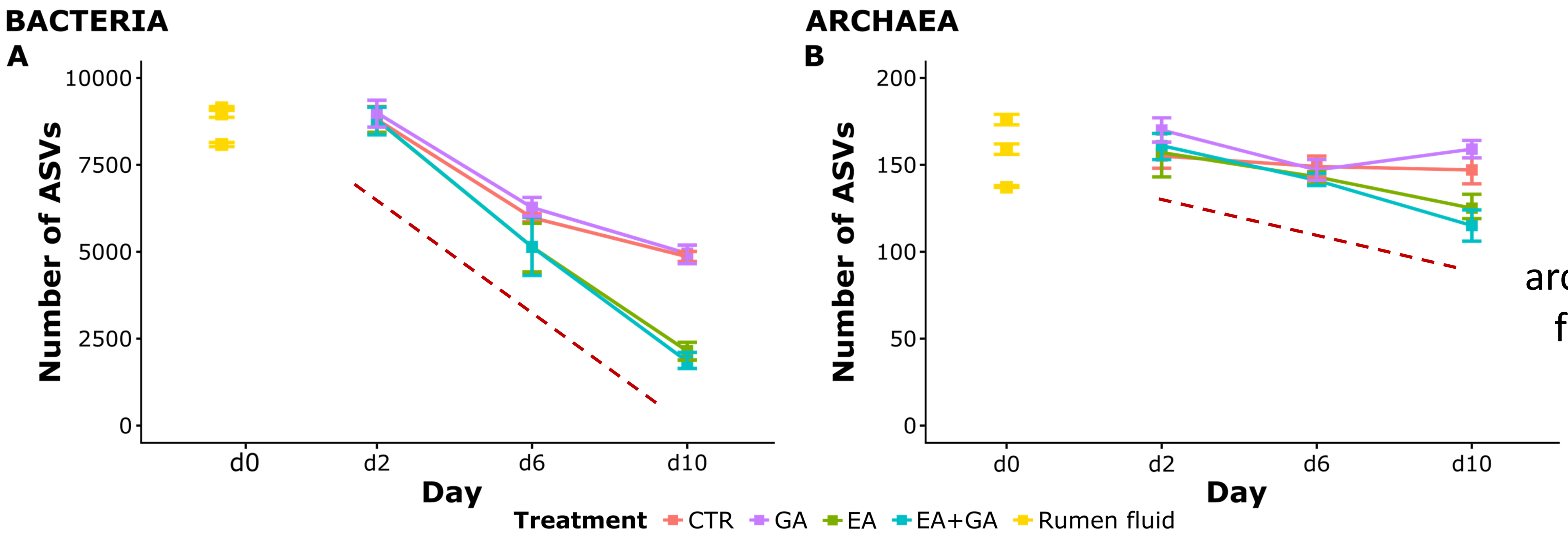


Stronger effects on bacterial community structures

Stronger effects with EA treatments

Results

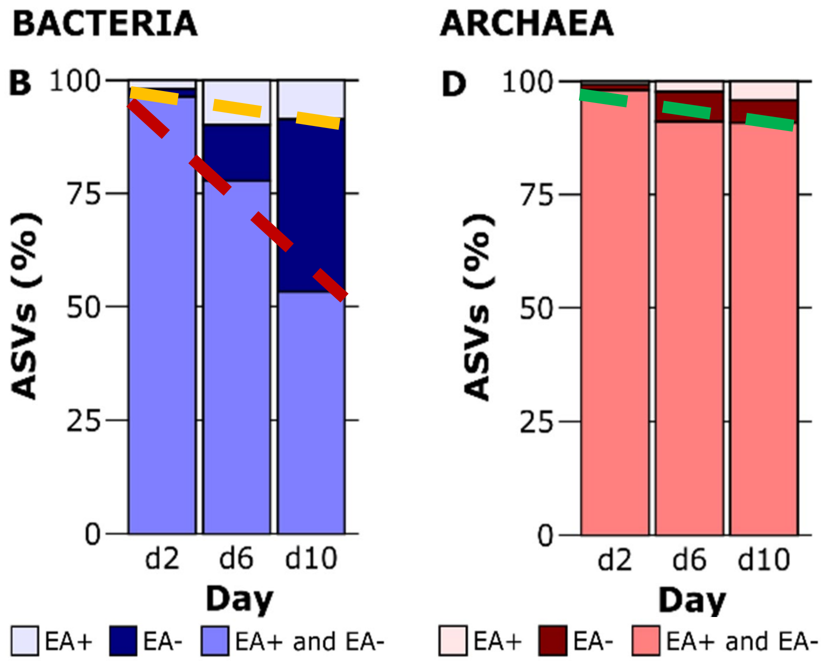
Microbial richness



Bacterial richness decreased more than archaeal richness over time following EA and EA+GA

Results

ASV distribution



Overall decreased % of **shared bacterial ASV** among EA+ and EA- (from 96% to 53%)

Relative increase of **bacterial ASV** in EA- treatments (Control and GA) (from 2% to 38%)

Smaller decrease for **shared archaeal ASV** (from 98% to 91%)

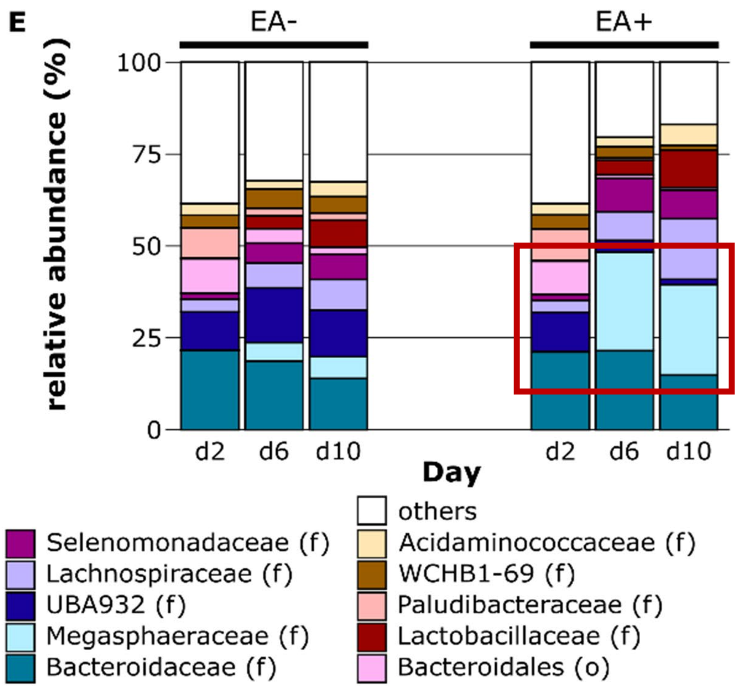
Which microbial groups have changed the most?

Taxonomic analysis

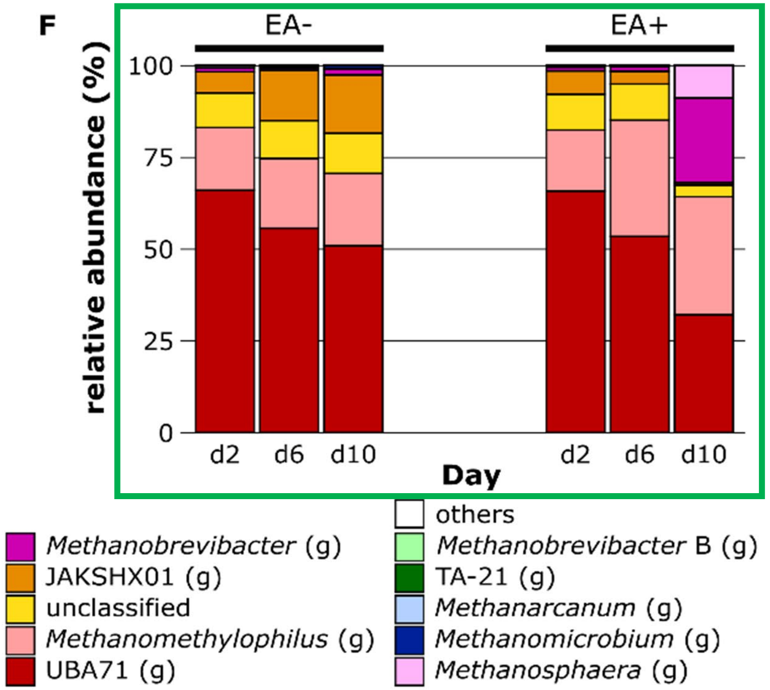
Taxonomic analysis

Results

BACTERIA



ARCHAEA

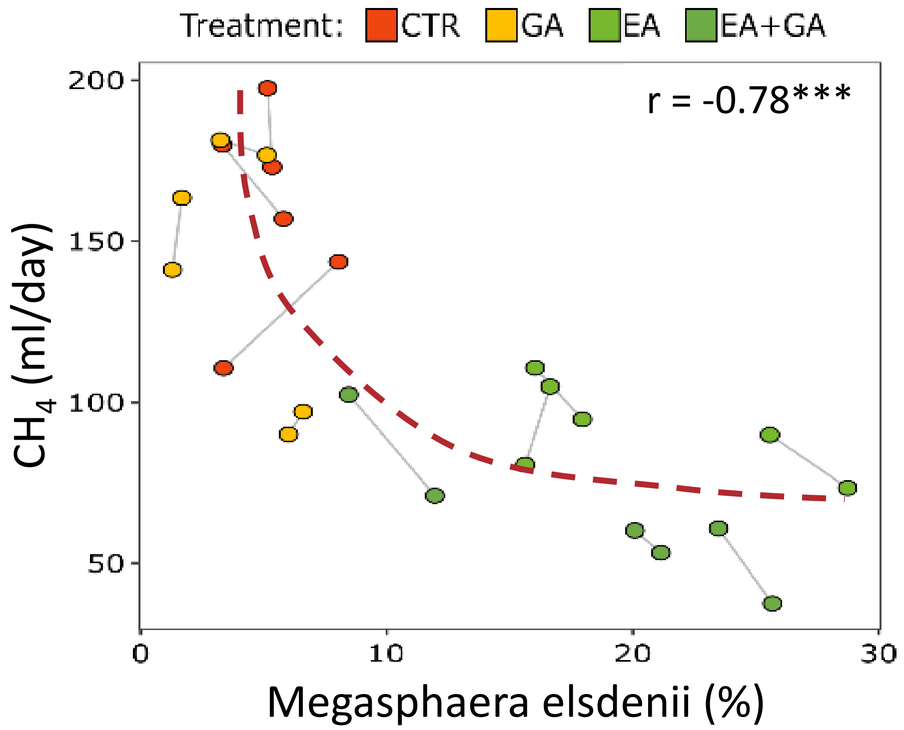


Increase of bacterial Megasphaeraceae in EA treatments

Modulation of archaeal methylotrophic Methanomethylophilaceae in all treatments

Taxonomy analysis

Results



- *Megasphaera elsdenii:*
  - Dominant bacterial species in both EA+ and EA- treatments, but…
  - Relative abundance at d10 higher in EA+ (19%) than EA- (5%)
  - Strongest negative correlation with CH_4 production (r = -0.78)

Increase of bacterial Megasphaeraceae in EA treatments

M. elsdenii → Lactate-consuming bacteria able to redirect H_2 to other H_2 sinks different from CH_4 (e.g. pyruvate or propionate)

Less H_2 available for methanogenesis → lower CH_4 production

Conclusions

- EA more effective than GA on rumen microbial composition and CH_4 production
- Bacterial communities more affected than archaeal communities
- Indirect effect on CH_4 → reduced H_2 available for methanogenesis

Open points

YES

Is EA a valid supplement to reduce CH_4?

NO

- Balance positive and negative effects of EA before *in vivo* application
- Elucidate the role of *M. elsdenii*

Lower CH_4 production
Modulation of rumen microbial community
Negative correlation of *M. elsdenii* with CH_4

Lower feed fermentation
Lower SCFA production

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Dr. Franco Widmer
Dr. Florian Gschwend

AgroVet-Strickhof (Lindau):
Dr. Melissa Terranova
Dr. Sergej Amelchanka

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Thank you

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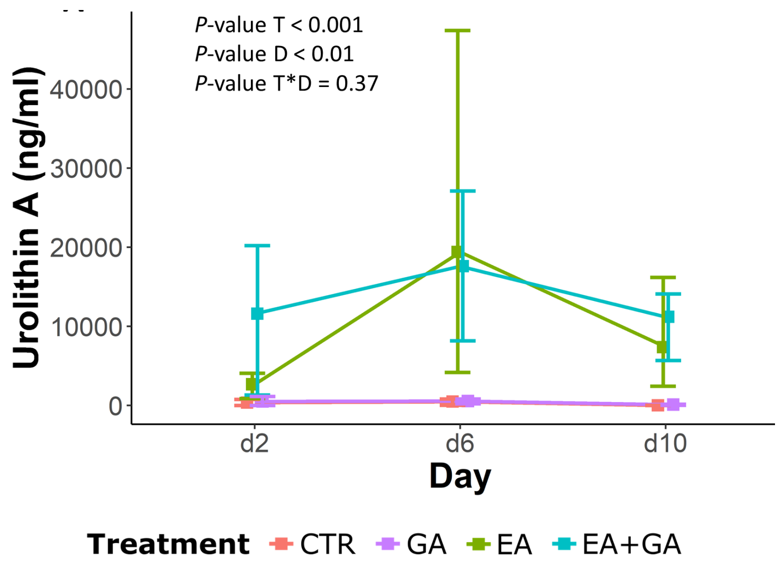
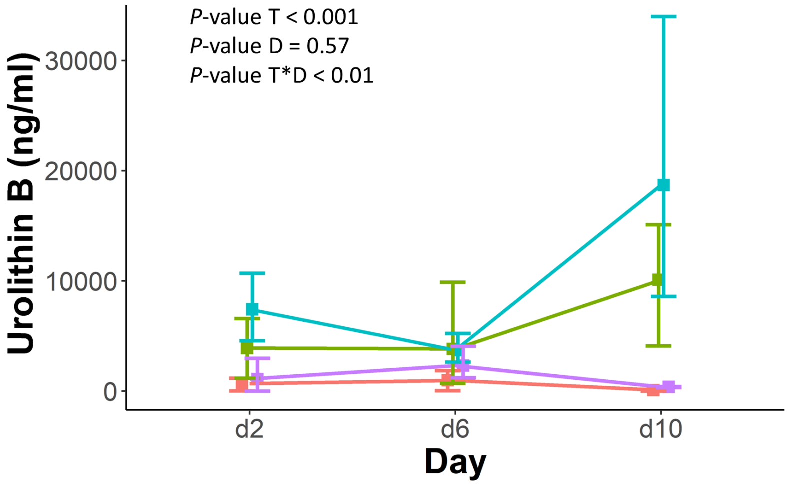
- Basal diet → 10 g DM of ryegrass hay and barley concentrate (7.5:2.5 ratio)
- Nutrient composition of basal diet (g/kg DM): 964 OM, 36 ash, 109 CP, 376 CF, 751 NDF, 417 ADF, 19 EE
- Donor cows fed 17.8 kg DM/day of a total mixed ration (TMR) composed of (% DM) grass silage (48%), maize silage (20%), sugar beet pulp (17%), hay (8%), concentrate (8%), and mineral supplement (0.2%).

Results



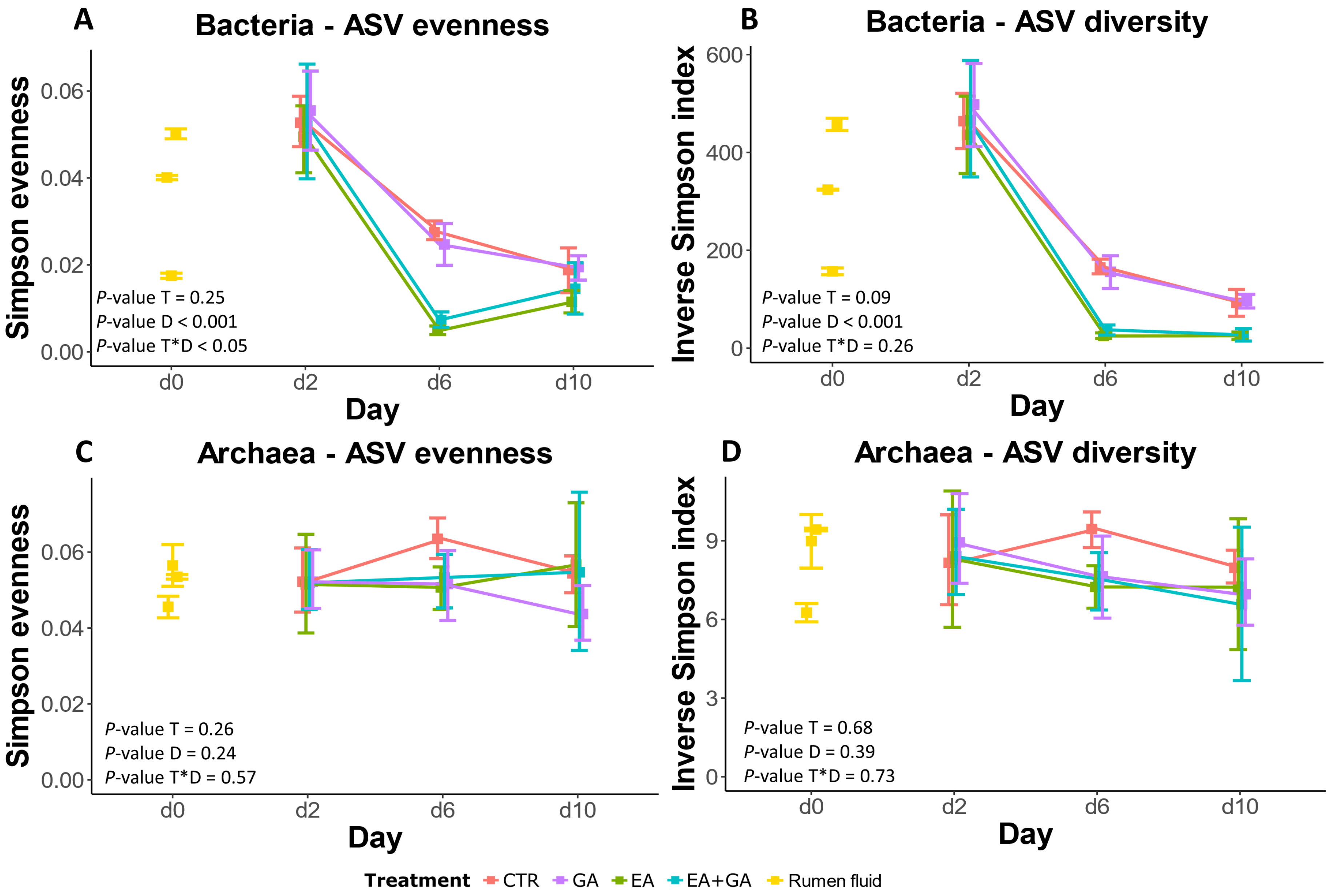


➢ HT secondary metabolites associated with metabolism of ellagitannins

- UroA → increased at d6 and d10 by EA and EA+GA
- UroB → increased at d10 by EA and EA+GA

UroA might have been converted to Uro B from d6 to d10

Involvement of metabotypes A, B, 0 based on urolithins produced by microbial communities, but so far only characterized in humans


A
Bacteria - ASV evenness
Simpson evenness
0.06
0.04
0.02
0.00
P-value T = 0.25
P-value D < 0.001
P-value T*D < 0.05
d0
d2
d6
d10
Day
B
Bacteria - ASV diversity
Inverse Simpson index
600
400
200
0
P-value T = 0.09
P-value D < 0.001
P-value T*D = 0.26
C
Archaea - ASV evenness
P-value T = 0.26
P-value D = 0.24
P-value T*D = 0.57
D
Archaea - ASV diversity
9
6
3
0
P-value T = 0.68
P-value D = 0.39
P-value T*D = 0.73
Treatment
CTR
GA
EA
EA+GA
Rumen fluid