Sea-derived microalgae leads to healthier red meat and reduced methane emissions

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Ruminant Sector Challenges

- Production
- Health
- Product Quality
- Environment

Interconnected
Rumen Microbiome Central to the Challenges

Bacteria
$10^9$-$10^{10}$/mL

Protozoa
$10^4$-$10^6$/mL

Fungi
$10^3$-$10^4$/mL

Archaea (methanogens)
Approx. $10^4$/mL

The rumen microbiome in action
Rumen fermentation

- Cellulose/hemicellulose to Volatile fatty acids: Energy source but source of released H.
- Volatile fatty acids: Acetate, lactate, butyrate, succinate, propionate
Food Security

FAO predict meat and dairy production will have to increase by 76% and 63% respectively by 2050
Methane and Productivity

Data has shown that ruminants which release less methane are more productive.

Some dietary interventions to reduce methane may increase productivity.
Ruminants and Methane

- FAO state that livestock agriculture responsible for approx. 18% of GHG emissions, mainly in the form of methane.

- Paris agreement: Limit global warming to less than 2%. A 45% reduction in methane emissions could reduce global warming by 0.3°C.

- Recent US-EU climate pledge to reduce methane emissions by 30% by 2030.
Cross-Sectorial GHG emissions

- Fossil fuel industry are the greatest producers of CO$_2$ which remains in the atmosphere for over 100 years.

- Livestock mainly produce methane which has a half life of less than 10 years.

- Irrespective need to decrease environmental impact of livestock by 2050.

THE MASTER PLAN:
Microbiome Applications for Sustainable food systems through Technologies and Enter prise

Project Co-ordinator:
Prof Paul Cotter, Teagasc, Ireland

http://www.master-h2020.eu/

@MASTER_IA_H2020 #MASTER
Microbiome Applications for Sustainable Food Systems through Technologies and EnteRprise

MASTER takes a global approach to the development of microbiome products, foods/feeds, services or processes with high commercial potential.

This will benefit society through improving the quantity, quality and safety of food across multiple food chains. These include marine, plant, soil, rumen, meat, brewing, fruit and vegetable waste, and fermented foods.

The MASTER project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 818368
Rumen microbiome

WP3: Rumen Microbiome – Improving animal production and reducing environmental impact through manipulation of the rumen microbiome Lead QUB (Huws), Co-Lead CSIC (Yanez-Ruiz), partners Teagasc, LUKE, INRA, Devenish, ICBF, AFBI,

Objective:

• Utilise host genomics to alter the rumen microbiome and animal phenotype.
• Define the rumen microbiome (different life stages) to improve efficiency, reduce greenhouse gases emissions & improve the fatty acid content of meat and milk.
• Develop microbiome-based tools to predict animal phenotype.
WP5: Integrated Microbiome Technologies for the Food Chain Lead UNITN (Segata), Co-lead QUB (Creevey), partners WU, Teagasc, UCC, 4DC, ULE, ONT, FFOQSI, Novolyse, Qiagen, Baseclear, Danone in close interaction with WPs 1, 2, 3, 4 and 6

WP5 will provide the technological, computational, and analytical tools to (i) support the other WPs, (ii) establish standardized tools and procedures for companies in the food chain, (iii) meta-analyse the produced data, and (iv) build user- and company-friendly resources to support all the microbial tasks associated with the food chain.
WP5: Integrated Microbiome Technologies for the Food Chain

SOPs

- SOP for validated sample collection and storage
- SOP for validated 16S rRNA-based microbiome processing
- SOP for validated shotgun metagenomics based microbiome processing

Pipelines/Tools

- MASTER database
- Public databases
- Assembly-free taxonomic and functional profiling
- Assembly-free strain profiling and tracking
- Assembly-based pipeline
- Antimicrobial resistance profiling
- Virome/microbiome profiling
- Statistical/ML pipelines

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WP3: Objectives

3.1: Utilise host genomics to beneficially alter the rumen microbiome and consequently animal phenotype.

3.2: Employ novel feeding technologies to define the rumen microbiome at different stages of life to improve efficiency, reduce greenhouse gas emissions and improve the fatty acid content of meat and milk.

3.3: Develop microbiome-based tools and mathematical models to predict animal phenotype.
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

3.2.1: Feed technologies in adult animals
2) Microalgae (link to WP 2)

50:50 isoenergetic diet (grass silage:concentrate)

Microalgae supplementation

Control
- 0% supplementary microalgae (0g DHA)

Low
- 1.2% supplementary microalgae (3g DHA)

Medium
- 2.4% supplementary microalgae (6g DHA)

High
- 3.6% supplementary microalgae (9g DHA)

56/24 Finishing Texel X Scottish black face lambs

33 days

56 animals
- 24 Chambers trial
- 32 Feed trial

Before and after samples
- Methane
- Oral swab
- Blood
- Ruminal liquid
- Urine (digestibility)
- Faeces (digestibility/ microbiome)

Slaughter samples
- Ruminal liquid
- Small intestine
- Large intestine
- Cecum
- Meat (2 and 7 days): loins, shoulder, legs

Image source: https://biorender.com

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3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

Animal Performance, digestibility and methane emission

- The DMI tended to decrease ($P=0.078$) in High treatment compared to the other diets ($n=24$).
- Digestibility of DM, OM, NDF, ADF and Energy were greater ($P \leq 0.030$) in Low and High treatment than in Control and Medium treatment, while N digestibility and retention were similar ($P \geq 0.325$) between treatments ($n=24$).
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

- No differences (P≥0.453) between Control and Microalgae-fed lambs were observed for initial body weight (BW) (n=56). Differences in BW were seen at different time points (see arrows below).
- The average daily gain (ADG) was greater (P=0.020), and the relative growth tended (P=0.068) to be greater in Low than in Medium treatment (n=56).

\[ \text{Relative growth} = \frac{W_2 - W_1}{(W_1 + W_2)/2} \times 100; \ W_1 = \text{Initial BW}, \ W_2 = \text{Final BW} \]

P-trt < 0.001; P-tim < 0.001; P-int = 0.004
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

Animal Performance, digestibility and methane emission

- Ruminal pH was lower (P=0.024) in Medium and High treatment than in Low treatment.
- Total VFA concentration was greater (P=0.012) in High than in Control and Low treatment.
- Methane emissions and methane/DMI were similar (P≥0.143) between treatments, however an approx. 8% reduction was seen with High treatment.

![Rumen pH](image1)

![Methane emission](image2)

![Total VFA concentration](image3)

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3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

- **Tendency for acetate to be lower** in lambs fed medium and high levels of microalgae.

- Whilst not significant, **modest increases in propionate** were also seen in lambs fed medium and high levels of microalgae.

![Indirect open-circuit respiration calorimeter chambers](image.png)

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<th>Control</th>
<th>Low</th>
<th>Medium</th>
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<tr>
<td>Acetate</td>
<td></td>
<td></td>
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<tr>
<td>%</td>
<td>64.42</td>
<td>63.41</td>
<td>58.29</td>
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- $P=0.078$

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<td>Butyrate</td>
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<td></td>
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<tr>
<td>%</td>
<td>13.02</td>
<td>14.02</td>
<td>15.17</td>
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- $P=0.157$

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<tbody>
<tr>
<td>Propionate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>19.42</td>
<td>20.43</td>
<td>22.50</td>
<td>23.16</td>
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- $P=0.533$
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

- DHA concentration increased in the loins as the microalgae content on the lamb's diet increased (P<0.05).
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

- **Microbiology testing (food standards):**
  ALS: Presumptive Coliforms; *Escherichia coli*; Thermotolerant *Campylobacter*; Aerobic colony count; β-Glucuronidase + *E. coli*; *Salmonella*; *Listeria* spp.

- Fatty acid concentration increased during the cooking (water loss of 37.8%)

**Home-sensorial trial in course**

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3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

- The average number of reads per sample after quality control was 23,553,087
- A total of 4712 metagenome associated genomes (MAGs) were extracted and characterized, functionally and taxonomically with an average number of 1,955 ORFs and average N50 metric was 23,937.

Abundance percentages across samples of the most abundant genera

Shotgun metagenomic sequencing

Illumina Nova seq 6000 S4 300 flow cell
(150bp PEat ~>6.2GB/ sample).
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

No differences seen in Archaea

Control treatment  Medium treatment
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

No differences seen in Archaea

- Control treatment
- High treatment

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3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

Higher abundances of many of these enzymes families when lambs fed medium levels of microalgae
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

CAZyme abundances

Higher abundances of some of these enzymes families when lambs fed high levels of microalgae
3.2: Dietary manipulations of the rumen microbiome for improved animal phenotype.

3.2.1: Feed technologies in adult animals

2) Microalgae oil (link to WP 2)

50:50 isoenergetic diet (TMR)

Microalgae supplementation

Control
0 DHA/TMR (g/kg DM) Microalgae oil

Low
2 DHA/TMR (g/kg DM) Microalgae oil

Medium
4 DHA/TMR (g/kg DM) Microalgae oil

High
6 DHA/TMR (g/kg DM) Microalgae oil

68 animals
24 Chambers trial
44 Feed trial

Before and after samples
- Methane
- Oral swab
- Blood
- Ruminal liquid
- Urine (digestibility)
- Faeces (digestibility/ microbiome)
- Diet (fatty acid/microbiome)

Slaughter samples
- Ruminal liquid
- Small intestine
- Large intestine
- Cecum
- Meat (2 and 7 days): loins, shoulder, legs

Image source: https://biorender.com
Conclusion

• Feeding freeze-dried microalgae at low, medium and high levels had no detrimental affect of animal health.

• Feeding freeze-dried microalgae at low, medium and high levels significantly increased DHA content of meat and burgers (pre and post cooking) with a small reduction in methane emissions (8%) seen following feeding on 4.5g/day of the microalgae (High)- human health benefits.

• Modest reductions seen in methane emissions (8%) likely due to changes in the bacterial fraction leading to the tendencies towards beneficial volatile fatty acid changes.

• No major effects on animal productivity.

• Gastrointestinal tract microbiome analysis ongoing with initial interesting data.

• Microalgae oil experiments ongoing.
Ruminant Sector Challenges

Production

Health

Product Quality

Environment

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The MASTER Consortium

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Thank you