In vitro incubation of dairy cow diets:
1. Degradability, total gas and methane yield

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Methane emissions

- CH$_4$ is one of fermentation gases (CO$_2$, CH$_4$, and VFA) → loss of gross energy intake to the animal, quantified around 2-12% (Johnson and Johnson, 1995)

- CH$_4$ is also considered an atmospheric greenhouse gas (GHG: CO$_2$, CH$_4$, N$_2$O, and O$_3$) → greenhouse effect & climate changes

- Assessments of CH$_4$ emissions vary considerably depending on the references and countries.
Objectives

✓ To evaluate *in vitro*
  - Degradability
  - Total gas production
  - Methane yield

of diets for dairy cows actually used in the Veneto region, in order to estimate their range of variation on methane emission
Diets

✓ A Reference Diet (RD) → representative of dairy cow diets commonly used in the Veneto region

✓ Other 7 diets were formulated changing the proportions of CP, NDF and lipids, within the limits of viable:

- Low Protein (LP)
- High Protein (HP)
- Low Fibre (LF)
- High Fibre (HF)
- Low Lipid (LL)
- High Lipid, fat supplement (HLF) (calcium soaps of palm)
- High Lipid, extruded oilseeds (HLO)

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### Chemical composition (g/kg DM) of 8 diets

<table>
<thead>
<tr>
<th></th>
<th>RD</th>
<th>LP</th>
<th>HP</th>
<th>LF</th>
<th>HF</th>
<th>LL</th>
<th>HLF</th>
<th>HLO</th>
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<tr>
<td>CP</td>
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<tr>
<td>NDF</td>
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<td>Lipids</td>
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<td>33</td>
<td>33</td>
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<td>Starch</td>
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<td>333</td>
<td>203</td>
<td>329</td>
<td>151</td>
<td>282</td>
<td>246</td>
<td>231</td>
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<tr>
<td>NFC</td>
<td>393</td>
<td>434</td>
<td>350</td>
<td>438</td>
<td>307</td>
<td>403</td>
<td>372</td>
<td>371</td>
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<tr>
<td>Ash</td>
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<td>46</td>
<td>60</td>
<td>46</td>
<td>60</td>
<td>52</td>
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<td>GE, MJ/Kg DM</td>
<td>18.6</td>
<td>18.5</td>
<td>18.7</td>
<td>18.7</td>
<td>18.7</td>
<td>18.4</td>
<td>19.1</td>
<td>19.1</td>
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<td>ME\text{TDN}, MJ/kg DM</td>
<td>12.1</td>
<td>12.2</td>
<td>11.8</td>
<td>12.7</td>
<td>11.1</td>
<td>11.8</td>
<td>12.7</td>
<td>12.7</td>
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</tbody>
</table>

- Low Protein (LP) and High Protein (HP)
- Low Fibre (LF) and High Fibre (HF)
- Low Lipid (LL) and High Lipid: fat supplement: calcium soaps of palm (HLF) and High Lipid: extruded oilseeds (HLO)

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## Experimental design

<table>
<thead>
<tr>
<th>Parameter</th>
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<tr>
<td>Diets</td>
<td>8</td>
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<tr>
<td>Incubation times</td>
<td>2</td>
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<td>Runs for each incubation time</td>
<td>2</td>
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<tr>
<td>Replications of diets for run</td>
<td>5</td>
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<tr>
<td><strong>Total bottles</strong></td>
<td>160</td>
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<td>Blanks</td>
<td>20</td>
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<tr>
<td>Temperature, °C</td>
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<tr>
<td>Feed sample, g</td>
<td>1.0 ± 0.010</td>
</tr>
<tr>
<td>Buffer, mL</td>
<td>100</td>
</tr>
<tr>
<td>Rumen fluid, mL</td>
<td>50</td>
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</tbody>
</table>
Automated GP system: Ankom® RF

- Kit of 50 bottles (317 ml), with spout and screw cap with septum puncture-proof, equipped with:
  - a pressure detector
  - an open-closed valve for gas venting at 6.8 KPa
- All bottles are wireless connected to a PC
- Pressure values inside the bottles are recorded by PC every minute

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Gas samples collection

✓ 9 mL of fermentation gas were collected with a gas-tight syringe and injected into a Vacuette®
✓ An aliquot of 10 μL was analyzed for CH₄ by GC
Computations

- NDF (NDFd, %) and true dry matter (TDMd, %) degradability (Goering and Van Soest, 1970)

- Gas production (GP, mL/g DM) was calculated by a conversion of psi into mL (Lopez et al., 2007)

- Total CH₄ production = CH₄ measured in bottle headspace × [(bottle headspace (257 mL) + GP mL)]

CH₄ loss was calculated according to Ramin and Huhtanen (2012)
Statistical analysis

✓ ANOVA (PROC GLM, SAS):
  ~ Diet (1-8)
  ~ Incubation time (24 and 48 h)
  ~ Run within incubation time (1-2)

✓ Contrasts:
  ~ LP vs HP diet → effect of CP level
  ~ LF vs HF diet → effect of NDF level
  ~ LL vs (HFL + HOL)/2 → effect of lipid level
  ~ HLF vs HLO → effect of lipid source
Effect of diet on NDFd (% NDF)

RD  LP  HP  LF  HF  LL  HLF  HLO

***P<0.001  * P<0.05

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Effect of diet on CH$_4$ (mL/g DM incubated)

***$P<0.001$
Effect of diet on CH$_4$ (mL/g TDM degraded)

**RESULTS & DISCUSSION**

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Take home messages

- The amount of *in vitro* CH$_4$ produced was comparable to that reported by the literature.

- The effects due to the CP, NDF and lipids were significant and comparable to other studies.

- The magnitude of these effects is small in actual diets for lactating dairy cows.

- In addition to N excretion, low-protein diets should also reduce CH$_4$ emission without negative effects on degradability of diet. This effect should be more carefully considered *in vivo*. 
Acknowledgements

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THANKS FOR YOUR ATTENTION

FOR MORE INFORMATION:
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## Ingredients (g/kg DM) of 8 diets

<table>
<thead>
<tr>
<th></th>
<th>RF</th>
<th>LP</th>
<th>HP</th>
<th>LF</th>
<th>HF</th>
<th>LL</th>
<th>HLF</th>
<th>HLO</th>
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<td>375</td>
<td>281</td>
<td>430</td>
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<tr>
<td>Alfalfa hay</td>
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<td>156</td>
<td>23</td>
<td>134</td>
<td>89</td>
<td>89</td>
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<tr>
<td>Ryegrass hay</td>
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<td>231</td>
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<td>Meadow hay</td>
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<td>47</td>
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<td>-</td>
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<td>60</td>
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<td>Corn meal</td>
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<td>Barley meal</td>
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<td>100</td>
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<td>Soybean meal</td>
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<td>24</td>
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</tr>
</tbody>
</table>

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Incubation procedures

✓ Rumen fluid was collected by an esophageal probe

✓ Buffer solution was prepared according to Menke and Steingass (1988)
Gas chromatograph

- Model 7820A GC system, Agilent Technologies
- Flame ionization detector
- 30 m stainless steel column, GS-CarbonPLOT, Agilent Technologies
- Gas carrier: Hydrogen, flow rate: 1.6 ml/min
- Isothermal oven temperature: 40°C
Effect of diet on GP (mL/g TDMd)

***P<0.001
** P<0.01

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Effect of diet on CH$_4$ (mL/100 mL GP)

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Effect of variation in CP, NDF and lipid content on CH₄ reduction

A decrease of 1% of chemical compound in the diet:

<table>
<thead>
<tr>
<th></th>
<th>mL CH₄ / g TDMd</th>
<th>mL CH₄ / g DM</th>
<th>KJ CH₄ / MJ GE</th>
<th>Ramin and Huhtanen (2013) In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP</td>
<td>-0.55</td>
<td>-0.41</td>
<td>-0.79</td>
<td>n.s.</td>
</tr>
<tr>
<td>NDF</td>
<td>-0.21</td>
<td>+0.33</td>
<td>+0.63</td>
<td>-0.46</td>
</tr>
<tr>
<td>Lipid</td>
<td>+0.55</td>
<td>+0.45</td>
<td>+0.86</td>
<td>+1.30</td>
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