Use of CO$_2$ as a breath marker

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When to use a marker?

A marker is often used when you are interested in a quantity but only a fraction is collected and analysed.

Well known examples:
- \( \text{Cr}_2\text{O}_3 \) in faeces for digestibility studies
- \( \text{SF}_6 \) in breath for methane production

Maybe better known in the future?
- \( \text{CO}_2 \) in breath for production of methane and other substances

Calculation:
Outbreath of the substance =
Concentration of a substance of interest in the breath (L/L) / concentration of the marker in breath (L/L)
* release of marker to breath (L/day) (Can be \( \text{SF}_6 \) or \( \text{CO}_2 \))
Methane is high on the present agenda but other substances can be of interest

• Acetone (ketosis)
• NO (lung infection)

(Phillips et al. 1999 mention more than 100 potential substances in human breath)

In the following I will focus on CO₂ and CH₄
**Why can CO$_2$ be used as a breath marker?**

Respiration experiments, including measurements of CO$_2$, O$_2$ and to a limited extent CH$_4$ has been done for 100 years. The first Danish publication based on the measurements was by H. Møllgaard and A.C. Andersen in 1917.

The measurements were done to be able to measure/predict the energy content of feeds and the energy requirements of animals.
Why is CO₂ a useful marker?

The CO₂ production and hence the breath of CO₂ can be predicted with the same precision as the energy requirements of the animals.

We do not need to use special designed and harmful markers as SF₆.

The marker is always at available.

Measurements can be made on whole herds in addition to measurements on individual animals.
How to calculate the quantitative CO$_2$ production?

**Maintenance**

- Intake of C
- Breath C
- CO$_2$
- CH$_4$
- Heat
- Feaces C + Urine C

- Metabolizable energy all becomes heat
- CO$_2$ can be calculated from heat production
Relation between heat and CO$_2$ production

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Fat</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>kJ / L CO$_2$</th>
</tr>
</thead>
<tbody>
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<td>kJ / L CO$_2$</td>
<td>28</td>
<td>24</td>
<td>21</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>kJ / L CO2</th>
</tr>
</thead>
<tbody>
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<td>Diet 1</td>
<td>5</td>
<td>20</td>
<td>75</td>
<td>21.95</td>
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<tr>
<td>Diet 2</td>
<td>3</td>
<td>18</td>
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<td>21.75</td>
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<td>Diet 3</td>
<td>4</td>
<td>16</td>
<td>80</td>
<td>21.76</td>
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<tr>
<td>Diet 4</td>
<td>1</td>
<td>4</td>
<td>95</td>
<td>21.19</td>
</tr>
</tbody>
</table>
Example Wallaby (16 kg live weight)

Heat production for maintenance = \(385 \text{ kJ/kg}^{0.75}\)

(Munn and Dawson (2003)

Wallaby heat production:
\[
16 \text{ kg}^{0.75} = 8 \\
8 \times 385 = 3080 \text{ kJ/day}
\]

Wallaby CO\(_2\) production:
\[
3080 / 21.75 = 142 \text{ l CO}_2/\text{day}
\]

Measured CH\(_4\)/CO\(_2\) ratio in the stable where the Wallabies were in Zoo = 0.020 (Corrected sample, see later)

Wallaby CH\(_4\) production:
\[
142 \times 0.020 = 2.8 \text{ l CH}_4/\text{day}
\]
Respiration chamber for one wallaby
Close relation between the CH$_4$/CO$_2$ ratio and lost energy in CH$_4$ - with modifications

CH$_4$/CO$_2$ versus CH$_4$E of GE

- Calves ~45 bulls. 117-268 kg LW. Open cirquet. Thorbek 1980''
- Cows 39 Holstein. Spot. Madsen et al.
- Wallaby. 8. 16 kg LW. Open cirquit. Madsen et al.
Methane measurements are performed at different levels

The CO₂ as a marker is of special interest when:
• the whole herd, - in a stable -, is going to be measured
• many individual animals are going to be measured
How to get the breath sample of a single animal?

Breath:
CO2 = 38000 ppm  
CH4 = 1800 ppm

Air:
CO2 = 380 ppm  
CH4 = 1,8 ppm
How to get the values for the real pure breath sample of a single animal?

When we collect we only get a fraction of the breath, the rest is the surrounding air.

Example:
Correction for concentration of breath in sample:

The sample consists of 4500 ppm CO₂ and 300 ppm CH₄
The stable air consists of 800 ppm CO₂ and 20 ppm CH₄
The breath consists of 3700 ppm CO₂ and 280 ppm CH₄
The CH₄/CO₂ ratio in the pure breath is: 280/3700 = 0.076

We use samples containing down to 5% of breath
How to calculate the quantitative CO$_2$ production?

Production

Intake of C → \( \text{CH}_4 \) → Breath C → \( \text{CO}_2 \) → Heat → Milk C + Deposited C → Feaces C + Urine C

Heat = Metabolizable energy
- energy in milk
- energy deposited
Close relation between the CH$_4$/CO$_2$ ratio and lost energy in CH$_4$

-with modifications

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Conclusions

Several methods are used and are still being developed to measure the CH$_4$ production by ruminants.

The numbers they produce all have possible errors and some can be corrected for.

**Example:**
The CH$_4$/CO$_2$ ratio can be corrected for different milk production.

**In general:**
Methods where the individual feed intake is measured can only get values from relatively few cows. -- Too little for breeders?

Other methods needs to calculate the individual feed intake.
-- Nutrition scientists know how best to calculate the feed intake!
-- Breeding scientists know how to calculate genetic relations!

Cooperation will produce the best results.
Conclusions

Numbers for the quantitative CH\textsubscript{4} production is needed and the CO\textsubscript{2}- methode produce that. The methods that use an imperical relation to the CH\textsubscript{4} production also need these reference values.

Specific for the CO\textsubscript{2} method

CO\textsubscript{2} production has to be calculated. -- Nutrition scientists have the knowledge how best to do this.

The animals are in their natural environmamt.

Investigations can be made on whole animal houses. (ruminants and other animals)

- Testing the effect of different feeds, breeds, species and production systems
Conclusions

The CO2-method should be used to investigate the opportunities to use different substances in the breath to monitoring dairy herds, especially on health.

Cooperation will produce the best results
Thank you for listening

I wish you a nice evening and a pleasant trip tomorrow
FTIR infrared analyser
250 different gasses
Diurnal variation in CH₄ and CO₂ release

CH₄/CO₂
61 dairy cows in 5 days (mean +/- 1,96*S.E.)

Hour of day

New feed New feed