Anaerobic in vitro rumen incubation technique to simulate rumen function

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vitro - Ex vivo

DESCRIPTION

Method keywords

Rumen inoculum

rumen microbes

anaerobic fermentation

gas production

digestion

Scientific area keywords

Feed degradation

Fermentation (kinetics)
Enteric methane (mitigation)
Rumen degradable/undegradable protein
Biohydrogenation of unsaturated fatty acid
Rumen microbiology
Rumen health

Method description

The *in vitro* rumen fermentation technique is used to simulate the rumen function/fermentation. The rumen is the first compartment of the digestive tract of ruminants, in which feed - consumed by ruminants - is fermented by rumen microbes. For optimal fermentation, the rumen provides an anaerobic environment at constant temperature and pH, and ensures a good mixing. We mimic the above-mentioned rumen conditions externally in an incubation flask. Briefly, the test products and the simulated ruminant feed are weighed into the flasks, which are sealed and flushed with CO2 to create the anaerobic conditions. Afterwards, bicarbonate/phosphate-buffered rumen fluid is added into the flasks which are incubated at 39 °C in a shaking incubator for a prescribed duration (mostly for 24 h). The rumen inocula/microbes are obtained from cannulated or non-cannulated live ruminants. Gas and incubated liquid samples are collected at the end of the incubation period to determine gas composition and volatile fatty acids. Additionally, samples are collected for microbial analysis. The anaerobic *in vitro* rumen incubation technique is well established in our lab, and we perform various experiments using this method which can be customized according to the research question. This includes kinetics of feed fermentation, protein and fat degradation in the rumen, biohydrogenation of unsaturated fatty acids, enteric methane mitigating ability of feed/additive, etc.

Lab equipment

- Incubator;
- CO2 flushing system;
- Water bath;
- pH meter;
- Pressure transducer;

- Gas chromatography to analyze the gas composition and (volatile) fatty acid profile;
- Equipment related to feed (residue) characterization;
- Equipment needed for microbial analysis (qPCR machine, bead beater, etc.).

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Resembles in vivo rumen condition;
- Versatile applications;
- Low cost and simultaneous screening of a large number of treatments;
- Does not need a large number of live animals (only rumen inoculum donor animals);
- Ethically superior;
- Less time-consuming.

Challenges

- End products and wastes accumulate in the bottle which may influence rumen function (particularly for incubation periods exceeding 72h);
- Absorption of nutrients through rumen wall cannot be mimicked;
- Interaction with host is not mimicked.

Modifications

- Preservation of rumen inocula for future use is currently under investigation

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Gadeyne, F., De Ruyck, K., Van Ranst, G., De Neve, N., Vlaeminck, B., & Fievez, V. 2016. Effect of changes in lipid classes during wilting and ensiling of red clover using two silage additives on in vitro ruminal biohydrogenation. Journal of Agricultural Science, 154, 553–566.

Associated documents

effect-of-changes-in-lipid-classes-during-wilting-and-ensiling-of-red-clover-using-two-silage-additives-on-in-vitro-ruminal-biohydrogenation.pdf

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