



# The micro gas test – a small scale *in vitro* system for high throughput analysis

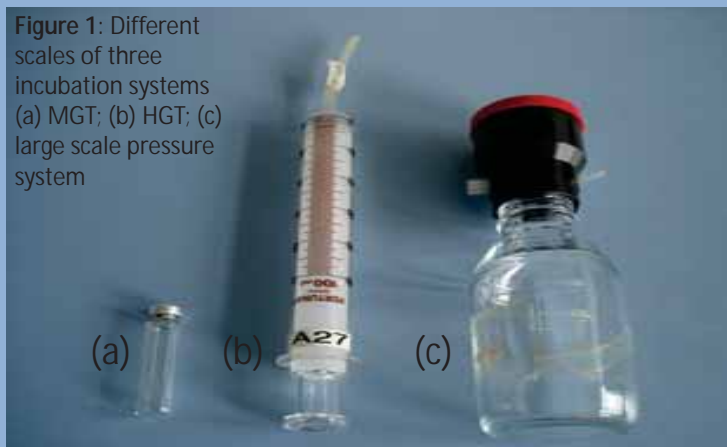
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## ABSTRACT

The newly developed micro gas test (MGT) is a small scale *in vitro* method, where gas production is measured through pressure increase. For the method's validation, a total of 14 feedstuffs, including two standard materials (hay and concentrate), were simultaneously incubated in the Hohenheim gas test (HGT) (Menke *et al.* 1979) and the MGT. There was strong correlation between the two methods. Compared to the HGT, the MGT gave maximum gas productions of nearly 90% for all feedstuffs investigated. Regression analysis of the mean values of gas production (mL/200 mg dry matter (DM)) from the MGT (y) and the HGT (x), respectively, resulted in the linear equation:  $y=0.88x+1.62$ ,  $R^2=0.99$ . **The results indicate that the MGT is a suitable method for *in vitro* feedstuff evaluations.**

Figure 1: Different scales of three incubation systems (a) MGT; (b) HGT; (c) large scale pressure system



## METHOD

- The MGT is a small scale *in vitro* method, conducted in 20 mL gas chromatography vials which serve as reactors for anaerobic digestion.
- 14 dried and ground feedstuffs were weighed into the vials, which were subsequently closed with a gas-tight sealing.
- Atmospheric conditions were adjusted by previous application of CO<sub>2</sub>.
- Samples were simultaneously incubated in the HGT (n=3) and in the MGT (n=4) at the same feedstuff-inoculate ratio.
- Incubation started with dispensing 5 mL inoculate and was carried out in a heating chamber (39°C). Ruminant fluid solution was prepared according to the HFT standard procedure.
- The relative pressure increase in the headspace was manually measured at predefined intervals (1,2,4,6,8,16,24h) and the headspace subsequently vented.
- In contrast to the HGT no continuous rotation but regular agitation was applied subsequent to pressure measurement in the MGT.

## RESULTS

- To display the measuring range of the feedstuffs within the two measuring systems replicate data points have been plotted (n=3, for MGT 3 out of 4 data point with maximum variances have been selected), see figure 2 on 2<sup>nd</sup> page
- Ranges vary for each feedstuff depending on the measuring system. Straw and (grass) hay standard samples, for instance, scatter more widely in the MGT than in the HGT. For rapeseed meal contrary results were obtained.
- In the MGT, material shape and texture of the feedstuffs as well as their floating characteristics influenced the ranges within replicate measurements.
- The MGT resulted in a significantly (p<0.05) lower 24 hour gas production as compared to the HGT (ca. 90%).
- Linear regression analysis of the 24 hour gas production data set using **replicate values** (n=3, maximum variances) resulted in a high R<sup>2</sup> of 0.97.
- For the **mean values** of the 24 hour gas production data set an **R<sup>2</sup> of 0.99** was calculated.
- Based on this high correlation between the two measuring systems, it is possible to convert the results of the HGT and the MGT directly using the linear equation **y=0.88x+1.62**.
- The relative dispersion of the measured values for both methods are <5%
- The mean value of coefficient of variation for the MGT is 2.7% compared to 1.5% for the HGT.



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## RESULTS CONTINUED

### Single data regression analysis of 24h gas production - HGT versus MGT -

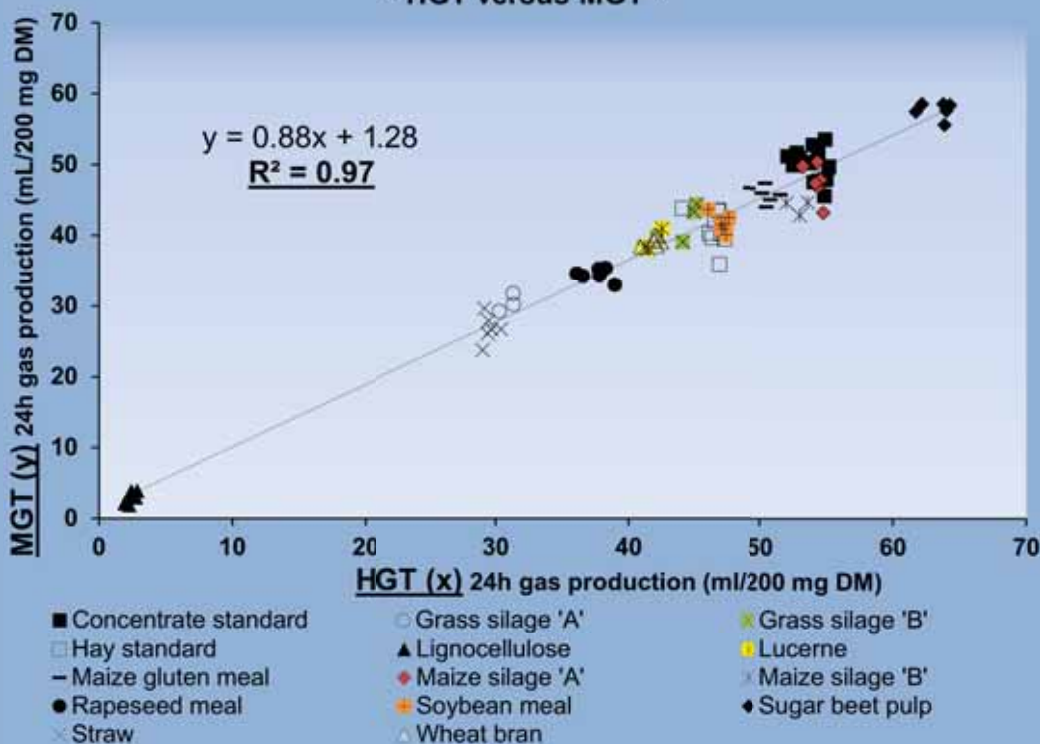


Figure 2: Regression analysis of the single data values of the micro gas test and the Hohenheim gas test (MGT = micro gas test, HGT = Hohenheim gas test, DM = dry matter)

## CONCLUSIONS

Regression analysis of the mean values of the well established and widely used HGT with the MGT resulted in a  $R^2$  value of 0.99. Thus, the MGT can be applied as an **alternative *in vitro* method** for feedstuff evaluation and the equation in the figure allows direct conversion of the MGT into the HGT results. To confirm this even more, additional feedstuffs, especially with low gas yields, will be included in the study, further on.

Furthermore, the MGT will be even more efficient as a tool for **high though put analysis**, e.g. during feed additive screenings, when it is carried out in an **automated system**, as it is part of the next study.



Figure 3: Automated micro gas test

## REFERENCES

Menke, K., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and the metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. Journal of Agricultural Science. 93, 217-222.

## AFFILIATIONS

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