doi:10.14720/aas.2024.120.1.16702 Original research article / izvirni znanstveni članek

Rumen gas kinetics: a comparative analysis of two *in vitro* assessment methods for forage evaluation

Nejc VALCL [1, 2], Andrej LAVRENČIČ [1]

Received November 22, 2023; accepted February 02, 2024.
Delo je prispelo 22. novembra 2023, sprejeto 02. februarja 2024

Rumen gas kinetics: a comparative analysis of two *in vitro* assessment methods for forage evaluation

Abstract: Gas production from thirty samples of feedstuffs (10 samples of corn silage, grass silage, and grass hay, respectively) was assessed *in vitro* using two methods: the Hohenheim gas test (HGT) and the ANKOM RF Gas Production System (ANKOM). Samples were incubated in buffered rumen fluid. Gas kinetic parameters were calculated using the Gompertz model. Results revealed significantly lower gas production with the ANKOM compared to the HGT. Significant differences were observed between the HGT and ANKOM in the specific gas production rate (parameter C), maximum fermentation rate (MFR) and gas produced after 24 h of incubation (Gas24) for each feed group. High coefficients of determination (R^2) were calculated between the methods for the gas kinetic parameters MFR, Gas24, total potential gas production (parameter B), decrease in the specific gas production rate (parameter A), moderate R^2 for C, and low R^2 for time of maximum fermentation rate (TMFR). Despite the lower quantities of gas generated with the ANKOM, there are strong correlations in the parameters of gas kinetics that promise the possibility of developing correction models. Future development of such models could position the ANKOM as a viable alternative to HGT, particularly for calculating metabolizable energy and net energy for lactation in feedstuffs.

Key words: animal nutrition, rumen, gas production, gas kinetic parameters, Ankom RF Gas Production System, Hohenheim Gas Test

Kinetika plinov v vampu: primerjalna analiza dveh *in vitro* metod za ocenjevanje krme

Izvleček: Tvorba plina iz tridesetih vzorcev krme (po 10 vzorcev koruzne silaže, travne silaže in sena) je bila ocenjena *in vitro* z dvema metodama: Hohenheimskim plinskim testom (HGT) in ANKOM RF Gas Production System (ANKOM). Vzorci so bili inkubirani v puferiranem vampovem soku. Parametri kinetike produkcije plinov so bili izračunani z uporabo Gompertzovega modela. Rezultati so pokazali statistično značilno manjšo tvorbo plina pri metodi ANKOM v primerjavi s HGT. Med metodama HGT in ANKOM smo opazili razlike v specifični hitrosti fermentacije (parameter C), največji hitrosti fermentacije (MFR) in plinu, proizvedenem po 24 urah inkubacije (Gas24), za vsako skupino krmil. Visoki koeficienti determinacije (R^2) med metodama so bili izračunani za MFR, Gas24, skupno potencialno tvorbo plina (parameter B) in faktor mikrobne (ne)učinkovitosti (parameter A), zmeren R^2 za parameter C in nizek R^2 za čas, v katerem je bila dosežena največja hitrost fermentacije (TMFR). Kljub manjšim količinam plina, ki je nastal z uporabo metode ANKOM, obstajajo močne korelacije v parametrih kinetike tvorbe plinov, ki kažejo na možnost razvoja korekcijskih modelov. S prihodnjim razvojem takih modelov bi bila metoda ANKOM lahko uporabljena kot zadovoljiva alternativa HGT, zlasti za izračun presnovljive energije in neto energije za laktacijo v krmi.

Ključne besede: prehrana živali, vamp, produkcija plinov, kinetika produkcije plinov, parametri, metoda ANKOM, Hohenheimski plinski test

1 University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia
2 Corresponding author, e-mail: nejc.valcl@bf.uni-lj.si

1 INTRODUCTION

In vitro measurements of gas production in the rumen are an important scientific method for estimating metabolizable energy (ME) and net energy for lactation (NEL) in conjunction with chemical analyses of feedstuffs. They are also used to determine the suitability of feed additives or feed rations for ruminants, the activity and biomass of microorganisms in the rumen and the quantification of volatile fatty acids produced during the incubation of substrata (Menke and Steingass, 1988; Aiple et al., 1995). The standard method for measuring *in vitro* gas production in the rumen is the Hohenheim gas test (HGT; Menke and Steingass, 1988). In this method, substrata are incubated in glass syringes and gas production is measured manually at fixed times. The method is therefore very labour-intensive. Since the development of the HGT, new methods have been developed that aim to automate and simplify the measurement process (Davies et al., 2000). One of the first such systems was developed by Theodorou et al. (1994), which measured gas production using a pressure transducer. The incubations were carried out in gas-tight culture flasks in which gases could accumulate during fermentation. The pressure in each flask was displayed on a digital indicator and then recorded manually. Excess gas that accumulated in each flask was removed manually with a syringe needle. Davies et al. (2000) then developed an automatic system where the gas that accumulates in the headspace of the flask during fermentation is automatically recorded and released when the pressure in the flask reaches a certain level. This was a direct improvement over the classic HGT system, as the researcher did not have to be present at certain times to manually record the amount of gas produced. For the accurate calculation of the gas kinetic parameters, it is important to record the gas volumes more frequently. This makes manual recording very labour intensive. Therefore, an automatic system such as the ANKOM^{RF} gas production system, which is able to automatically record the gas pressure produced at more frequent times during the incubation (e.g. every 30 minutes), should improve the accuracy and reliability of the measurements. The disadvantages of automatic systems are that they are prone to electronic and mechanical errors. Such a system is also more expensive than the manual HGT. For the correct estimation of rumen degradability of feedstuffs and the effects of feed additives, it is very important that the different systems are as accurate as possible when measuring rumen *in vitro* gas production.

A comparison of the manual (HGT) and automatic (ANKOM) methods of measuring rumen *in vitro* gas production was carried out to determine whether the automatic method is a valid replacement for the standard HGT. The aim of the study was to compare the HGT with the ANKOM based on the gas production measured with both methods. The hypothesis was that the ANKOM does not differ from the HGT in terms of gas production measurements and therefore kinetic parameters of *in vitro* gas production.

2 MATERIALS AND METHODS

2.1 SUBSTRATES AND CHEMICAL COMPOSITION

Thirty samples of forages, ten samples of varying quality of grass silage (GS), 10 of corn silage (CS) and 10 of grass hays (H), were used as substrates. The substrata were dried at 50 °C to the constant mass and ground through a 1 mm sieve. Samples were analysed for dry matter (DM), crude protein (CP), ash and ether extract (EE). Neutral detergent fibre (NDF) content was determined using the Van Soest method (Goering and Van Soest, 1970). Chemical compositions of each feed group are presented as means ± standard deviation in Table 1. All values are presented as g kg^{-1} DM unless specified otherwise.

2.2 EXPERIMENTAL DESIGN AND *IN VITRO* GAS PRODUCTION

For the evaluation of *in vitro* gas production, two measurement techniques were used: manual (HGT) with the measurement of the volume of gas in the glass syringes as described by Menke and Steingass (1988),

Table 1: Chemical composition of corn silages, grass hays and grass silages (arithmetic means ± standard deviations; n = 10 for each feed group)

Indices	Corn silages	Grass hays	Grass silages
DM (g kg^{-1})	940 ± 10.6	937 ± 11.8	928 ± 15.9
Ash	33 ± 3.5	70 ± 15.6	108 ± 38.1
EE	27 ± 3.1	15 ± 3.7	26 ± 6.0
CP	67 ± 11.9	95 ± 18.6	136 ± 33.2
NDF	465 ± 96.7	630 ± 45.3	533 ± 89.3
NFC	407 ± 101.0	190 ± 62.5	197 ± 110.2

DM – dry matter; EE – ether extract; CP – crude protein; NDF – neutral detergent fibre; NFC – non-fibre carbohydrates (1000 – (CP + EE + Ash + NDF))

and automatic with the measurements of gas pressure within the 100 ml glass flasks (ANKOM).

Rumen fluid was taken from two mature castrated Jezersko Solčavska × Romanovska rams (*Ovis aries*), with an average weight of 70 kg, fitted with permanent rumen cannula. A daily ration consisting of average quality hay (Ash = 38 g kg^{-1} DM, CP = 172 g kg^{-1} DM, NDF = 579 g kg^{-1} DM) was offered to them ad libitum (approx. 1.5 kg consumed) with the addition of 0.25 kg pelleted commercial compound feed (160 g CP kg^{-1}), and mineral and vitamin mix (0.025 kg) once per day. The diet composition was calculated according to the German metabolizable energy (ME) and utilisable protein requirements (nXP; DLG, 1997) with which the protein and energy requirements for maintenance were met and the energy-to-protein ratio of the rumen was balanced. Animals were kept in compliance with animal welfare regulations (U33401-12/2019/9 dated 16.7.2019, issued by the Food Safety, Veterinary and Phytosanitary Inspectorate, Ministry of Agriculture, Forestry and Food, Republic of Slovenia Food Safety, Veterinary and Plant Protection Administration, Ljubljana, Slovenia).

The study was conducted from October 2022 to March 2023 in 10 total consecutive runs (1 week = 1 run). All samples were incubated in rumen fluid using the HGT (runs = 3) and ANKOM (runs = 7). The number of consecutive runs for the ANKOM was higher due to the smaller number of ANKOM modules available. With both methods, the buffer medium was prepared as described by Menke and Steingass (1988) using the rumen fluid to buffer solution ratio of 1:2. Both methods included two blank samples (only inoculum without substrate) and two samples of Italian ryegrass (*Lolium multiflorum*) 2nd cut in flowering period as hay standard. Gas production after 24 h for the hay standards was known (HGT: 196 ml 1 g^{-1} DM^{-1}; ANKOM: 140 ml g^{-1} DM^{-1}). Hay standard factors (measured/known gas production) were then calculated between runs and ranged from 0.92 to 1.12. The mean standard factor for this trial was 1.004, hence gas production measurements for each run were not corrected.

Sheep rumen fluid was taken before morning feeding, and was transported to the laboratory immediately inside a thermo flask heated to 39 °C, and strained through two and then four layers of cheesecloth. Using the manual method, gas production kinetics were evaluated by anaerobically incubating each feed sample (250 ± 5 mg/syringe) in four 100 ml glass syringes filled with 30 ml of buffered rumen fluid. Syringes were kept in a water bath at 39 °C. Gas production was measured manually after 0, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h. The syringes were manually shaken at each measurement. If gas production exceeded 80 ml in the first 36 h, the volume was recorded and the gas was released. In each run, two blank samples and two hay standard samples were included.

Using the automatic method, developed by Ankom Technology® (Macedon, NY, USA; ANKOM^{RF} gas production system), each feed sample (250 ± 5 mg/flask), was anaerobically incubated in the 30 ml of buffered rumen fluid. Each unit consisted of a 100 ml glass flask (actual volume: 137 ml; headspace volume: 107 ml) and an ANKOM pressure sensor module, equipped with a microchip and a radio sender. The system automatically measures gas pressure inside the unit and automatically releases the pressure when it reaches a set threshold of 7.5 kPa. The decision to set the pressure threshold to 7.5 kPa was based on methodologies of studies using the ANKOM method for *in vitro* gas production measurements (Tagliapietra et al. 2010; Cornou et al. 2013; Bachmann et al. 2020). The gas pressure was recorded every 30 minutes for 72 h. After the start of the incubation, the flasks were manually shaken daily. After incubation, the gas pressure was converted into amount units (moles) of gas using the "ideal" gas law (Equation 1) and then converted to millilitres (ml) of gas produced by Avogadro's law (Equation 2):

$$n = p\left(\frac{V}{RT}\right) \qquad (1)$$

where n is gas produced in moles (mol), p is cumulative pressure in kilopascals (kPa), V is the headspace volume in the glass flask in litres (l), T is the temperature in Kelvin (K) and R is gas constant (8.314472 J $(K \times mol)^{-1}$). The gas production is then calculated as:

$$GP\ (ml) = n\ \times 22.4\ \frac{l}{mol} \times 1000\ \frac{ml}{l} \qquad (2)$$

where GP is the volume of gas produced.

2.3 CALCULATIONS AND STATISTIC ANALYSES

Gas production kinetic parameters were calculated as described by Lavrenčič et al. (1997). The net volume of gas produced at each incubation time was calculated as the difference between the total volume of gas produced and the volume of gas produced from the blank sample at each time of incubation. Net volumes at each time of incubation were adjusted afterwards to 1 g of substrate DM. The obtained *in vitro* gas production data were then fitted with the Gompertz model (Lavrenčič et al., 1997):

$$Y_t = B \times e^{-C \times e^{-At}} \quad (3)$$

Where Y_t is gas produced at the time "t" (ml g^{-1} DM), B is the total potential gas production (ml g^{-1} DM), C is the specific gas production rate, A is the decrease in specific gas production rate and t is the time in hours (h).

The parameters were calculated in SAS 9.4. (SAS Software ver. 9.4; SAS Institute, Cary, NC, USA), using the PROC NLIN procedure for a nonlinear regression method with the Marquardt compromise to estimate the kinetic parameters and fit the curve for each syringe within a substrate. By inserting a fixed time of 24 h in the equation of the Gompertz model with known parameters, the amount of gas produced in 24 h was calculated. By setting the second derivative of the Gompertz model to zero (0) and solving for "t", times of maximum fermentation rates (TMFR) were calculated:

$$\frac{d^2y}{dt^2} = A \times B^2 \times C^2 \times (e^{-At})^2 \times e^{-C \times e^{-At}} - A \times B \times C^2 \times e^{-C \times e^{-At}} = 0 \rightarrow TMFR \quad (4)$$

Using the corresponding value of TMFR in the first derivative equation, maximum fermentation rates (MFR) were calculated:

$$MFR = B \times C \times A^{-A \times TMFR \times e^{-C \times e^{-A \times TMFR}}} \quad (5)$$

With a one-way analysis of variance (ANOVA), using the general linear model (PROC GLM), the effect of the feed group, sample, method and interaction between the feed group and method on the estimated and calculated kinetic parameters of gas production, was compared. The results are presented as least square means (Table 1):

$$Y_{ijkl} = \mu + F_i + S_j + M_k + FM_{ik} + e_{ijkl} \quad (6)$$

Where Y_{ijkl} are the estimated and calculated kinetic parameters of gas production, F_i is the effect of the feed group (i = corn silage, grass silage, grass hay), S_j is the effect of the sample (j = 1, 2… 30), M_k is the effect of the method (k = ANKOM, HGT) and FM_{ik} is the interaction between feed groups and methods.

3 RESULTS AND DISCUSSION

Within each feed group, significant differences ($p < 0.05$) were found between the ANKOM and HGT for the parameters C, MFR and Gas24, but not for B, A and TMFR (Table 2). Parameter C measured with ANKOM was 25 % lower for maize silage, 27 % lower for grass silage and 38.5 % lower for grass hay compared to the HGT. Using the ANKOM, the MFR parameter was lower in every of the feed groups compared to the HGT. The differences were –41.3 % for corn silages, –50 % for grass silages and –62.8 % for grass hays, respectively. The rate at which gas is produced with the ANKOM

Table 2: Effect of method, individual sample, and feed group on estimated and calculated kinetic parameters of *in vitro* gas production

Feed group	Method	B (ml g^{-1} DM)	C	A	MFR (ml h^{-1})	TMFR (h)	Gas24 (ml g^{-1} DM)
Corn silage	ANKOM	240	2.11	0.096	8.4	7.9	191
	HGT	259	2.99	0.166	14.3	6.6	242
Grass silage	ANKOM	160	1.97	0.087	5.3	8.6	120
	HGT	182	2.69	0.156	10.6	6.3	168
Grass hays	ANKOM	177	1.63	0.049	2.9	12.5	97
	HGT	206	2.56	0.102	7.8	9.5	162
		P – values					
F		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
S		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
M		<.0001	<.0001	<.0001	<.0001	0.0005	<.0001
F × M		0.6470	0.0168	0.0625	<.0001	0.5486	0.0082

F – feed group; S – sample; M – method; F × M – interaction between feed group and method; B – total potential gas production; C – specific gas production rate; A – decrease in specific gas production rate; MFR – maximum fermentation rate; TMFR – time of maximum fermentation rate; Gas24 – gas produced after 24 h of incubation

could be affected by the amount of gas dissolved in the medium. With the ANKOM, the gas produced is released out of the headspace of the flask only when the pressure inside reaches a certain threshold. According to Henry's law, a certain proportion of these gases are dissolved in the medium and are released into the headspace of the flask only after the excess gasses are already released (Theodorou et al., 1994). It has also been pointed out that microbial activity could be disturbed, if the pressure exceeds 48 kPa (Theodorou et al., 1994). Lowman (1998) implied that continuous venting of flasks at 4.5 kPa should prevent the saturation of the solution. They also found out that, if the flasks were not shaken at all, their gas production was higher than in flasks shaken recurrently at pre-set times or continuously on an orbital shaker. Supersaturation of CO_2 in the medium may also occur at high gas pressures in closed systems, which can lead to biased measurements of gas production (Tagliapietra et al. 2010; Cattani et al. 2014). In our study, the threshold for venting the ANKOM flasks was set at 7.5 kPa, which was similar to Cornou et al. (2013), and the flasks were shaken regularly. With the HGT, the plungers of the syringes were greased to avoid high pressures by allowing the accumulated gases to expand, and they were shaken at fixed times.

Gas produced after 24 h for feeds measured with the HGT (Table 2) was similar to the findings of Getachew et al. (2004). Their corn silage produced 232 ml g^{-1} DM gas, while their wheat silage, which was similar to our grass silages, produced 172 ml g^{-1} DM gas. Compared to HGT, the ANKOM resulted in significantly lower ($p < 0.05$) gas production in the first 24 h

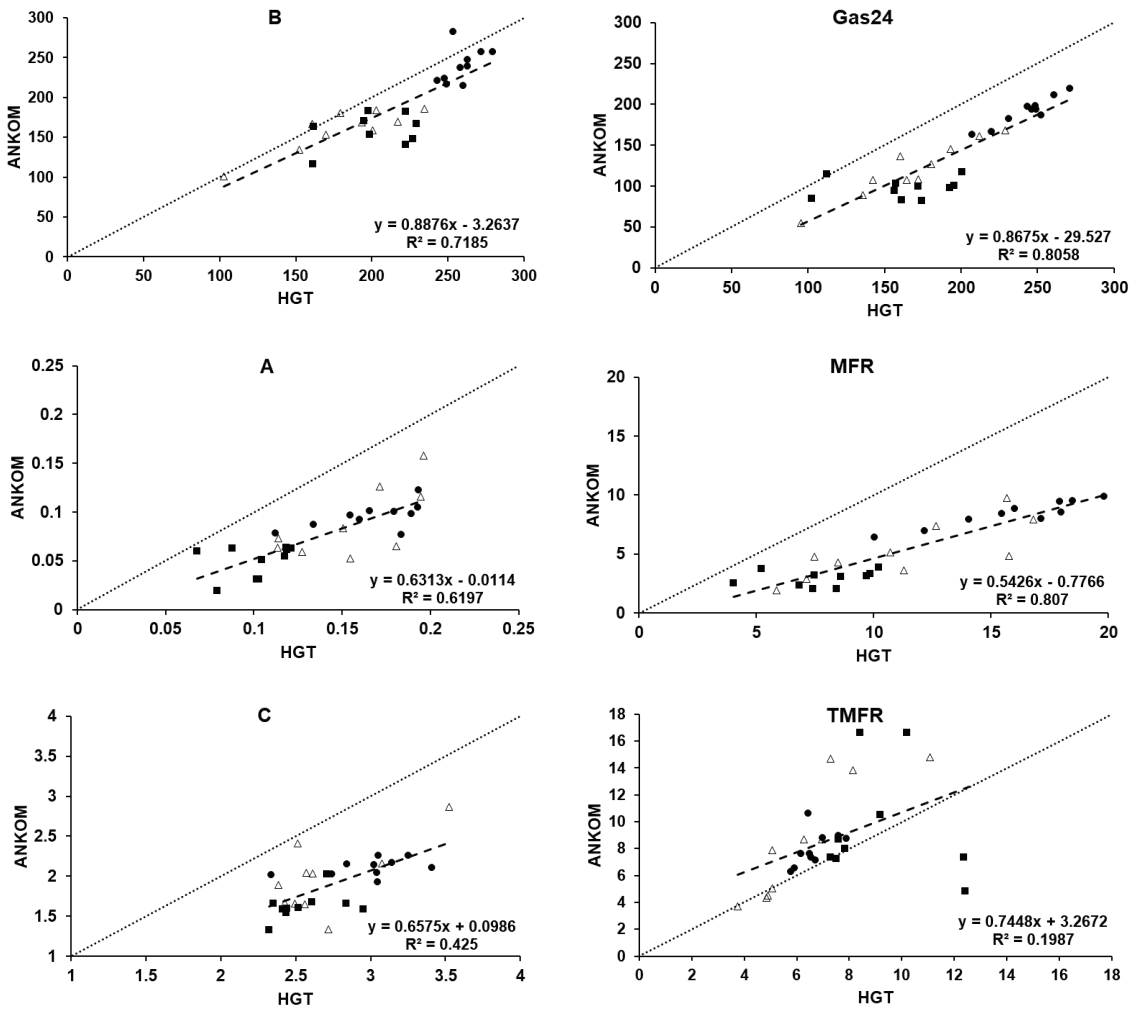

Figure 1: Relationship between parameters B, Gas24, A, MFR, C, and TMFR using the HGT (Hohenheim gas test) versus the ANKOM RF gas system (dashed line = regression line; dotted line = constant diagonal) for corn silage (●), grass hays (■) and grass silage (Δ)

of incubation (Gas24) for all feed groups. The differences were –21.0 % for corn silages, –28.5 % for grass silages and –40.1 % for grass hays. Bachmann et al. (2020) reported lower gas production measured in general with the ANKOM compared to the HGT. They also reported that the rankings of substrates remained the same regardless of the system used. These results were similar to our findings, as corn silages produced the most gas, grass silages were ranked second and grass hay produced the least gas, in both the HGT and ANKOM system. Our results also partly agree with Gierus et al. (2008), who have shown that their automated pressure evaluation system produced significantly less gas after 24 h compared to HGT for grass silages but not for grass hay, while Elberg et al. (2018) have shown, that gas production in 24 h of incubation in the automated system compared to HGT, was significantly lower for corn silages and for grass hays, but not for grass silages. Gas production can be affected by the ratio between rumen fluid and buffer, the diet of donor animals, and the general variability of rumen fluid on collection day (Rymer et al., 2005). In this study, the incubation parameters were standardised across both methods, except in times of rumen fluid collection. Due to the smaller number of ANKOM modules available, we performed the *in vitro* incubation in a higher number of runs than with the HGT. Considering all these factors, we cannot fully explain the differences between the compared systems.

Figure 1 shows the distribution of parameters for each sample comparing the ANKOM to the HGT. All the single values for each parameter were plotted. The coefficients of determination (R^2) show a strong correlation between the methods across all samples in the parameters B, A, MFR, and Gas24, a moderate correlation for parameter C, and a very weak correlation for parameter TMFR. Bachmann et al. (2020) reported an R^2 of 0.57 for gas produced after 24 h (Gas24) between the two methods from substrates used, however, they used a smaller number of substrates (n = 6), compared to our study (n = 30).

4 CONCLUSIONS

The gas kinetic parameters C, MFR and Gas24 obtained with the ANKOM differed significantly from the HGT. Despite these differences, the rankings for each feed group in gas produced and gas kinetic parameters were equal between both methods and showed strong correlations between the methods in all parameters except TMFR. In this regard, the methods are comparable in terms of ranking, but not in terms of absolute values. For the calculation of ME and NEL, with the results obtained from the ANKOM, caution should be taken as there are significant differences between the methods in the Gas24 parameter. It is also necessary to be aware of the difference in gas production when using the ANKOM and to reference the method used when listing the results in the study. Despite the lower quantities of gas generated *in vitro* with the ANKOM compared to the HGT, there are strong correlations in the parameters of gas kinetics that show the possibility of developing correction models. With the development of correction models in the future, the ANKOM could become a valid replacement for the HGT for calculations of gas kinetic parameters and more importantly, for calculating ME and NEL of feedstuffs.

5 REFERENCES

Aiple, K. P., Steingass, H., Drochner, W. (1995). Schätzung des Energiegehaltes von Grunfutteramitteln mit Pepsin-Cellulase-methode und dem Hohenheimer Futterwerttest. *Proceedings of the Society of Nutrition Physiology*, *4*, 99.

Bachmann, M., Kuhnitzsch, C., Martens, S. D., Steinhöfel, O., Zeyner, A. (2020). Estimation of gas production and post-ruminal crude protein from native or ensiled Pisum sativum and Vicia faba grains. *Journal of Applied Animal Research*, *48*(1), 78–85. https://doi.org/10.1080/09712119.2020.1733581

Cattani, M., Tagliapietra, F., Maccarana, L., Hansen, H. H., Bailoni, L., Schiavon, S. (2014). Technical note: *In vitro* total gas and methane production measurements from closed or vented rumen batch culture systems. *Journal of Dairy Science*, *97*(3), 1736–1741. https://doi.org/10.3168/jds.2013-7462

Cornou, C., Storm, A. I., Hindrichsen, B. I., Worgan, H., Bakewell, C. E., Yáñez-Ruiz, D., … Hansen, H. (2013). A ring test of a wireless *in vitro* gas production system. *Animal Production Science*, *53*(6), 585–592. https://doi.org/10.1071/AN12091

Davies, Z. S., Mason, D., Brooks, A. E., Grif, G. W., Merry, R. J., Theodorou, M. K. (2000). An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. *Animal Feed Science and Technology, 83*(3–4), 205–221. https://doi.org/10.1016/S0377-8401(99)00138-8

DLG (Deutsche Ladwirtschafts-Gesellschaft), (1997). Feed Value Table for Ruminants. 7th Revised and Extended Edition (in German: DLG Futterwerttabellen: Wiederkäuer). DLG-Verlag. Frankfurt (Germany)

Elberg, K., Steuer, P., Habermann, U., Lenz, J., Nelles, M., Südekum, K.-H. (2018). A small scale *in vitro* system for high throughput gas production analysis – A comparison with the Hohenheim gas test. *Animal Feed Science and Technology*, *241*, 8–14. https://doi.org/10.1016/j.anifeedsci.2018.04.001

Getachew, G., Robinson, P. H., DePeters, E. J., Taylor, S. J. (2004). Relationships between chemical composition, dry matter

degradation and *in vitro* gas production of several ruminant feeds. *Animal Feed Science and Technology*, *111*(1–4), 57–71. https://doi.org/10.1016/S0377-8401(03)00217-7

Gierus, M., Schiborra, A., Südekum, K.-H., Rave, G., Taube, F. (2008). Comparison of gas accumulation profiles of several feeds using manual or automated gas production methods. *Animal Feed Science and Technology*, *147*(4), 310–325. https://doi.org/10.1016/j.anifeedsci.2008.02.001

Goering, H. K., Van Soest, P. J. (1970). Forage Fiber Analysis (Apparatus Reagents, Procedures and Some Applications). Agricultural Handbook No. 397. United States Department of Agriculture, Washington DC.

Lavrenčič A., Stefanon, B., Susmel, P. (1997). An evaluation of the Gompertz model in degradability studies of forage chemical components. *Animal Science, 64,* 423–431. https://doi.org/10.1017/S1357729800016027

Lowman, R. S., Brooks, A. E., Theodorou, M.-K., Dhanoa, M. S., Cuddeford, D. (1998). The effect of head-space pressure on gas production profiles as determined using the pressure transducer technique. *In vitro* techniques for measuring nutrient supply to ruminants (ed. Deaville, E. R., Owen, E., Adesogan, A. T., Rymer, C., Huntington, J. A., Lawrence, T. L. J.), pp. 205–206. British Society of Animal Science occasional publication no. 22. https://doi.org/10.1017/S0263967X00032602

Menke K. H., Steingass H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Development, 28*, 7–55.

Rymer, C., Huntington, J. A., Williams, B. A., Givens, D. I. (2005). *In vitro* cumulative gas production techniques: History, methodological considerations and challenges. *Animal Feed Science and Technology*, *123–124*, 9–30. https://doi.org/10.1016/j.anifeedsci.2005.04.055

Tagliapietra, F., Cattani, M., Bailoni, L., Schiavon, S. (2010). *In vitro* rumen fermentation: Effect of headspace pressure on the gas production kinetics of corn meal and meadow hay. *Animal Feed Science and Technology*, *158*(3–4), 197–201. https://doi.org/10.1016/j.anifeedsci.2010.04.003

Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., France, J. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, *48*(3–4), 185–197. https://doi.org/10.1016/0377-8401(94)90171-6