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RESEARCH ARTICLE

Estimation of Partial Gas Production Times of Some Feedstuffs Used in Ruminant Nutrition

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Summary

The aim of this study was to determine the gas production kinetics of wheat straw, alfalfa hay and barley grain and estimate *t25, t50, t75* and *t95* using $Y = A(1\text{-exp}^{-ct})$ exponential model. Gas productions were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times. At all incubation times gas production of barley grain was significantly ($P<0.01$) higher than those of wheat straw and alfalfa hay. The *in vitro* gas production rate (c) and total gas production (A) of barley grain was significantly ($P<0.01$) higher than those of wheat straw and alfalfa hay. Time to produce 25, 50, 75 and 95% of total gas production (*t25, t50, t75* and *t95*) of barley grain also were significantly ($P<0.01$) lower than those of wheat straw and alfalfa hay. As a result, in addition to the *"c"* and *"A"*, using $Y = A(1\text{-exp}^{-ct})$ exponential model the estimation of t25, t50, t75 and t95 will provide more useful data to compare feedstuffs in terms of *in vitro* fermentation studies.

Keywords: *In vitro gas production, Partial gas production times, Wheat straw, Alfalfa hay, Barley grain*

Ruminant Beslemede Kullanılan Bazı Yemlerin Kısmi Gaz Üretim Zamanlarının Tahmini

Özet

Bu çalışmanın amacı, buğday samanı, yonca otu ve arpa danesinin gaz üretim kinetiklerini, *t25, t50, t75* ve *t95* gibi kısmi gaz üretim zamanlarını $Y = A(1\text{-exp}^{-ct})$ üssel fonksiyonunu kullanarak belirlemektir. Gaz üretimi 0, 3, 6, 12, 24, 48, 72 ve 96 saatlerinde belirlenmiştir. Bütün inkübasyon zamanlarında arpa danesinden üretilen gaz miktarı, buğday samanı ve yonca otundan üretilen gazdan önemli ($P<0.01$) derecede daha fazla bulunmuştur. Arpa danesinin gaz üretim hızı *(c)* ve üretilen toplam gaz miktarı (A), buğday samanı ve yonca otunun gaz üretim hızlarından ve üretilen toplam gaz miktarından önemli ($P<0.01$) derecede daha fazla bulunmuştur. Arpa danesine ait kısmi gaz üretim zamanları (*t25, t50, t75* ve *t95*), buğday samanı ve yonca otuna ait kısmi gaz üretim zamanlarından önemli ($P<0.01$) derecede daha düşük bulunmuştur. Sonuç olarak, *"c"* ve *"A"* parametrelerine ilave olarak, $Y = A(1\text{-exp}^{-ct})$ üssel fonksiyonu kullanarak, kısmi gaz üretim zamanlarının (*t25, t50, t75* ve *t95*) hesaplanması, yemlerin *in vitro* fermantasyon açısından karşılaştırılması için daha fazla ve yararlı datalar sağlamıştır.

Anahtar sözcükler: *İn vitro gaz üretimi, , Kısmi gaz üretim zamanları, Buğday samanı, Yonca otu, Arpa danesi*

INTRODUCTION

Recently the *in vitro* gas production technique has been widely used to compare the ruminant feedstuffs. In this method the feedstuffs are subjected to fermentation in glass syringes or bottles with buffered rumen liquid. The gas produced during fermentation is determined at time intervals such as 3, 6, 12, 24, 48, 72 and 96 h. The gas production data at time intervals is fit to some mathematical models to obtain gas production kinetics such as gas production rate and extent. In addition to raw gas production data at time intervals, this gas production

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kinetics are widely used to compare the ruminant feedstuffs in terms of fermentation. ($Y = a + b(1 - \exp^{-ct})$ and $y = A - BQ^t Z^{\sqrt{t}}$ model are widely used to fit gas production data obtained at different time intervals using packet programs such as NEWAY, Graphpad, CurveExpert and Maximum Likelihood and FIG. P [1-6].

Although ($Y = a + b(1 - \exp^{-ct})$ exponential model was widely used to fit in situ degradation data it was suggested that the exponential model can be used to fit *in vitro* gas production data [1-3]. However, recently many researchers have preferred to choose ($Y = A(1 - \exp^{-ct})$ exponential model instead of ($Y = a + b(1 - \exp^{-ct})$ model. *"a"* values should be zero when "t" is equal to 0. There is no gas production since the fermentation of sample is not started [7-11]. However, this is not the case in practice. Generally positive or negative "a" values were obtained when ($Y = a + b(1 - \exp^{-ct})$ was used to fit the gas production data. Most of researchers have interpreted "a" values as gas production from the quickly soluble fraction of sample fermented. On the other hand, some researchers preferred to use the $y = A - BQ^t Z^{\sqrt{t}}$ instead of the exponential model Since Maximum Likelihood packet program allow the calculation t_{50} and t_{95} when gas production data is fitted to $y = A - BQ^t Z^{\sqrt{t}}$ model [5,12-14]. However, so far, most of researchers who used the exponential model have ignored the calculation of some parameters such as time (h) to produce 50% of total gas production (t_{50}) and time (h) to produce 95% of total gas production (t_{95}) although they are very important parameters to compare the feedstuffs using *in vitro* gas production [1,14-16]. So far, there is limited information about the calculation of the time (h) to produce %25, 50, 75 and 95 of total gas production (t_{25}, t_{50}, t_{75} and t_{95} respectively) when the exponential model is chosen to fit the gas production data.

Therefore the aim of this study was to determine the gas production kinetics of wheat straw, alfalfa hay and barley grain and estimate t_{25}, t_{50}, t_{75} and t_{95} using ($Y = A(1 - \exp^{-ct})$ exponential model.

MATERIAL and METHODS

In this experiment, total three feedstuffs (wheat straw, alfalfa hay and barley grain) which are the locally available and widely used feedstuffs in ruminant rations were chosen to obtain the gas production when they are subjected to fermentation *in vitro*. All chemical analyses were carried out in triplicate. Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method [17]. CP was calculated as N X 6.25. EE were determined by the method of [17]. Neutral detergent fiber (NDF) content was determined by the method of Van Soest *et al.*[18]. ADF and ADL contents were determined following the method of Van Soest [19].

Wheat straw, alfalfa hay and barley grain samples milled through a 1 mm sieve were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke *et al.*[20]. Rumen fluid was obtained from 1.5-2 years old three fistulated kivircik sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The water was given ad libitum. The sheep were kept in individual metabolic cages. 0.200 gram dry weight of samples was weighed in triplicate into calibrated glass syringes of 100 mL. The syringes were prewarmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. Readings of gas production were recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 h after incubation [20]. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to non-linear exponential model as: $Y = A(1-\exp^{-ct})$

Where *Y* is gas production at time't', *A* is the total gas production (ml/200 mg DM), *c* is the gas production rate constant (h^{-1}) and *t* is the incubation time (h).

"t_{25}"(time (h) to produce 25% of total gas production) was calculated as follows

$$Y = A(1 - \exp^{-ct_{25}})$$

$$\frac{\frac{25}{100}A}{A} = \frac{A(1 - \exp^{-ct_{25}})}{A}$$

$$\frac{25}{100} = (1 - \exp^{-ct_{25}})$$

$$\exp^{-ct_{25}} = 1 - \frac{25}{100}$$

$$\exp^{-ct_{25}} = \frac{75}{100}$$

$$-ct_{25} = \ln(0.75)$$

$$t_{25} = \frac{\ln(0.75)}{-c}$$

$$t_{25} = \frac{0.288}{c}$$

t_{50}, t_{75}, t_{95} were estimated in a similar way, putting 50/100, 75/100 and 95/100 values instead of 25/100 in the equation mention above. The equations for t_{50}, t_{75} and t_{95} were given below:

$$t_{50} = \frac{0.693}{c}, \quad t_{75} = \frac{1.386}{c}, \quad t_{95} = \frac{2.996}{c}$$

One-way analysis of variance (ANOVA) was carried out to determine the effect of feedstuffs type on gas production and their kinetics using General Linear Model of Statistica for Windows. Significance between individual means was identified using Tukey's multiple range tests. Mean differences were considered significant at (P<0.001). Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS

The proximate chemical composition of wheat straw, alfalfa hay and barley grain are given in *Table 1*. There are considerable variations among feedstuffs in terms of chemical composition.

The gas production at different time intervals is given in *Fig. 1*. At all incubation times gas production of barley grain was significantly higher than those of wheat straw and alfalfa hay.

The gas production kinetics of wheat straw, alfalfa hay and barley grain is given in *Table 2*. There are significant (P<0.01) differences among feedstuffs in terms of gas production kinetics. The *in vitro* gas production rate and extent of barley grain was significantly (P<0.01) higher than those of wheat straw and alfalfa hay. The t_{25}, t_{50}, t_{75} and t_{95} of barley grain also were significantly higher than those of wheat straw and alfalfa hay.

DISCUSSION

As can be seen from *Fig. 1* at all incubation time gas production of barley grain was significantly higher than those of wheat straw and alfalfa hay possibly due to high fermentable carbohydrate content of barley grain. It is well known that gas production is associated with volatile fatty acid (VFA) production following fermentation of substrate so the more fermentation of a substrate the greater the gas production, although the fermentation end

Table 1. *Chemical composition of wheat straw, alfalfa hay and barley grain*

Tablo 1. *Buğday samanı, yonca otu ve arpa danesinin kimyasal bileşimi*

Composition (g/kg DM)	Feedstuffs			SEM	Sig.
	WS	AH	BG		
CP	3.30c	15.6a	12.1b	0.227	***
Ash	6.5b	7.1a	2.5c	0.071	***
EE	2.0c	3.2c	2.3b	0.037	***
NDF	79.5a	44.1b	17.8c	0.918	***
ADF	54.3a	33.3b	10.7c	0.555	***
ADL	10.0a	11.2a	1.9b	0.661	***

*a b c Row means with common superscripts do not differ (P>0.05); **s.e.m.** - standard error mean; **Sig.** - significance level; **CP** - Crude protein, **EE** - Ether extract, **NDF** - Notral detergent fiber, **ADF** - Acid detergent fiber, **ADL** - Acid detergent fiber*

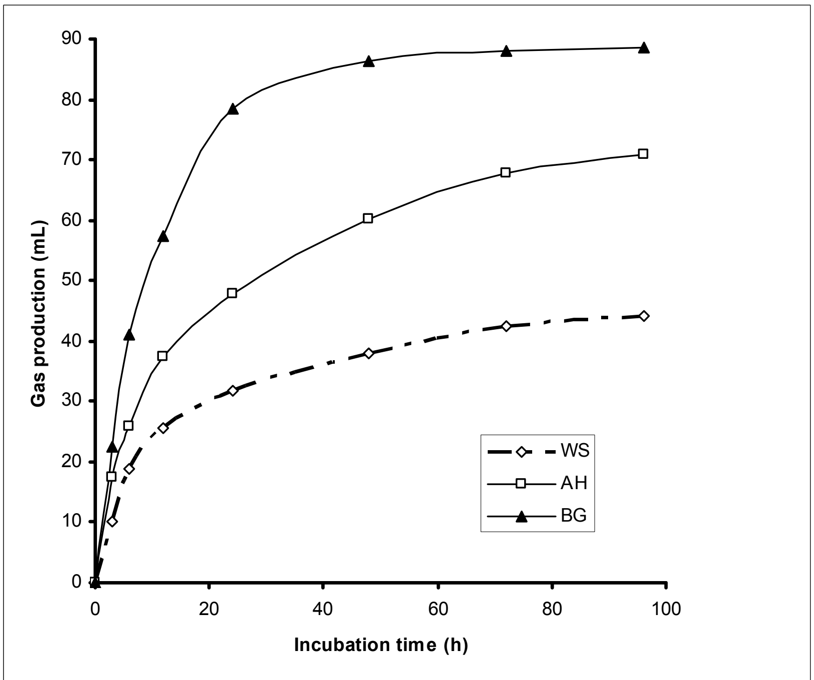

Fig 1. Gas production of wheat straw (WS), alfalfa hay (AH) and barley grain (BG)

Şekil 1. Buğday samanı, yonca otu ve arpa danesinin gaz üretimi

Table 2. *Gas production parameters of wheat straw, alfalfa hay and barley grain*

Table 2. *Buğday samanı, yonca otu ve arpa danesinin gaz üretim parametreleri*

Estimated Parameters	Feedstuffs			SEM	Sig.
	WS	AH	BG		
c	0.081b	0.065c	0.096a	0.002	***
A	41.37c	66.95b	87.66a	0.616	***
t_{25}	3.58b	4.38a	3.01c	0.100	***
t_{50}	8.61b	10.56a	7.24c	0.239	***
t_{75}	17.23b	21.11a	14.47c	0.475	***
t_{95}	37.25b	45.65a	31.29c	1.028	***

*[a b c] Row means with common superscripts do not differ (P>0.05); **s.e.m.** - standard error mean; **Sig.** - significance level; **c** - gas production rate (%); **A** - potential gas production (mL); t_{25} - time (h) to produce 25% of total gas production; t_{50} - time (h) to produce 50% of total gas production; t_{75} - time(h) to produce 75% of total gas production; t_{95} - time (h) to produce 95% of total gas production; *** P<0.001*

products do influence more closely with gas production [2]. Differences between total gas productions could be explained by the differences in total VFA production and molar proportion of VFA [21].

Generally the higher the gas productions rate the lower t_{25}, t_{50}, t_{75} and t_{95} will be obtained. The time (h) to produce a given gas production is positively correlated with gas production rate. As can be seen from Table 2 t_{25}, t_{50}, t_{75} and t_{95} of barley grain were significantly lower than those of wheat straw and alfalfa hay although barley grain had a significantly higher total gas production than those of wheat straw and alfalfa. The total gas production should be taken into consideration when feedstuffs are compared in terms of t_{25}, t_{50}, t_{75} and t_{95} values.

As a result, in addition to the "*c*" and "*A*", using ($Y = A(1 - \exp^{-ct})$) exponential model the estimation of t_{25}, t_{50}, t_{75} and t_{95} will provide more useful data to compare feedstuffs in terms of *in vitro* fermentation studies.

REFERENCES

1. Orskov ER, McDonald P: The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *J Agric Sci,* 92, 499-503, 1979.

2. Blummel M, Orskov ER: Comparison of an *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Anim Feed Sci Technol*, 40, 109-119, 1993.

3. Getachew G, Blummel M, Makkar HPS, Becker K: *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: A review. *Anim Feed Sci Technol,* 72, 261-281, 1998.

4. Kamalak A, Canbolat O, Gürbüz Y, Ozay O, Özköse E: Chemical composition and its relationship to *in vitro* gas production of several tannin containing trees and shrub leaves. *Asian-Austral J Anim Sci,* 18 (2): 2003-208, 2005.

5. Theodorou MK, Willams BA, Dhanoa MS, McAllan AB, France J: A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim Feed Sci Technol,* 48, 185-197, 1994.

6. Kamalak A, Gürbüz Y, Finlayson JH: Comparison of *in vitro* dry matter degradation of four maize silages using the Menke gas production method. *Turk J Vet Anim Sci,* 26, 1003-1008, 2002.

7. Kamalak A, Canbolat Ö, Özkan ÇÖ, Atalay Aİ: Effect of Thymol on *in vitro* gas production, digestibility and metabolizable energy content of alfalfa hay. *Kafkas Univ Vet Fak Derg,* 17 (2): 211-216, 2011.

8. Larbi A, Duguma B, Mollet M, Smith JW, Akinlade A: Edible forage production, chemical composition, rumen degradation and gas production characteristics of *Calliandra calothyrsus* (Messin) provenances in the humid tropics of West Africa. *Agroforest Syst,* 39 (3): 275-290, 1998.

9. Chaji M, Naserian AA, Valizadeh R, Mohammadabadi T, Mirzadeh KH: Potential use of high-temperature and low-temperature steam treatment, sodium hydroxide and an enzyme mixture for improving the nutritional value of sugarcane pith. *S Afr J Anim Sci*, 40 (1): 22-32, 2010.

10. Hervás G, Ranilla, MJ, Mantecón ÁR, Tejido ML, Frutos P: Comparison of sheep and red deer rumen fluids for assessing nutritive value of ruminant feedstuffs. *J Sci Food Agric*, 85 (14): 2495-2502, 2005.

11. Mohammadabadi T, Chaji M, Tabatabaei S: The effect of tannic acid on *in vitro* gas production and rumen fermentation of sunflower meal. *J Anim Vet Adv*, 9 (2): 277-280, 2010.

12. France J, Dhanoa MS, Theodorou MK, Lister SJ, Davies DR, Isaac D: A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *J Theor Biol*, 163, 99-111, 1993.

13. Lopez S, France J, Dhanoa SM, Mould F, Dijkstra J: Comparison of mathematical models to describe disappearance curves obtained using the polyester bag technique for incubating feeds in the rumen. *J Anim Sci*, 77, 1875-1888, 1999.

14. Kamalak A, Canbolat O, Gürbüz Y, Erol A, Ozay O: Effect of maturity stage on chemical composition, *in vitro* and *in situ* dry matter degradation of tumbleweed hay (Gundelia tournetortii L.). *Small Rum Res,* 58, 149-156, 2005.

15. Pell AN, Pitt ER, Doane HP, Schofield P: The development use and application of the gas production at Cornell university, USA. *Brit Soc Anim Sci,* 22, 45-54, 1998.

16. Macheboeuf D, Milgen VJ: Comparison of five models used to describe gas accumulation profiles in the gas test method with horse caecal fluid as inoculum. *in vitro* techniques for measuring nutrient supply to ruminants. *Brit Soc Anim Sci,* 22, 185-186, 1998.

17. AOAC: Official Method of Analysis. Association of Official Analytical Chemists. 15th ed., pp.66-88, Washington, DC. USA 1990.

18. Van Soest PJ, Robertson JD, Lewis BA: Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animals nutrition. *J Dairy Sci,* 74, 3583-3597, 1991.

19. Van Soest PJ: The use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. *JAOAC*, 46, 829-835, 1963.

20. Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W: The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J Agric Sci Camb*, 93, 217-222, 1979.

21. Beuvink JMW, Spoelstra SF: Interactions between substrate, fermentation end products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen micro-organisms *in vitro*. *Appl Microbiol Biotechnol*, 37, 505-509, 1992.