In vitro gas production measurements and estimated energy value and microbial protein to investigate associative effects of untreated or biological treated linen straw and berseem hay

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in

Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Nutritional and foraging ecology of sheep and goats

Zaragoza : CIHEAM / FAO / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85

2009
pages 261-266

In vitro gas production measurements and estimated energy value and microbial protein to investigate associative effects of untreated or biological treated linen straw and berseem hay

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Abstract. An *in vitro* gas production technique was used to investigate the associative effects of untreated or biologically-treated linen straw with berseem hay (H). The linen straw (LS) was biologically treated with white rot fungi (TLS). Ground samples (200 mg DM) of H, LS, TLS and the mixtures (50% w/w) of H + LS and H + TLS were incubated in 100 ml glass syringes with buffered rumen fluid. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and the kinetics of gas production was described by using the equation Gas (t) = b [1 – exp (–c (t – L))]. Gas production volume was highest for hay (P < 0.05) and greater for H + LS (P < 0.05) than LS and TLS. Total gas production at 96 h and rate of gas production increased when LS or TLS were mixed with hay. Metabolizable energy (ME) and net energy (NE) were higher (P < 0.01) for H, H + LS and H + TLS than for LS and TLS. Organic matter digestibility (OMD %) and microbial protein production were significantly higher for H, H + LS and H + TLS than for LS or TLS. In conclusion, positive associative effects on *in vitro* gas production occurred more consistently when untreated and biologically-treated linen straw was incubated in mixtures with hay.

Keywords. Gas production – Digestibility – Straw – Hay – Sheep.

Mesure de la production de gaz* in vitro*, énergie estimée et protéines microbiennes pour étudier les effets associatifs de la paille de lin traitée ou non traitée et du foin de Bersim

Résumé. *La technique de production de gaz* in vitro *a été utilisée pour étudier les effets associatifs de la paille de lin (LS) non traitée ou traitée et le foin de Bersim (H). La paille de lin (PL) a été biologiquement traitée avec un champignon (TLS). Des échantillons broyés (200 mg de matière sèche) de H, LS, TLS et les mélanges (50% p/p) de H + LS et H + TLS ont été incubés dans des seringues en verre de 100 ml. Le volume de gaz produit dans chaque seringue a été enregistré après 3, 6, 9, 12, 24, 48, 72 et 96 h d'incubation et les cinétiques de production de gaz ont été modélisées selon le modèle suivant : Production de gaz (t)* = b *[1 – exp (–*c (t – L*))].* Le *volume de gaz produit a été le plus élevé pour le foin (P < 0,05). Le substrat H + LS a produit plus de gaz (P < 0,05) que LS et TLS. Le volume total de gaz produit et la vitesse de production de gaz après 96 h d'incubation augmentent lorsque LS et TLS ont été mélangés avec le foin. L'énergie métabolisable (ME), l'énergie nette (NE), la digestibilité de la matière organique (OMD %) et la production de protéines microbiennes sont plus élevées avec H, H + LS et H + TLS qu'avec LS et TLS. En conclusion, les effets positifs sur la production de gaz* in vitro *sont obtenus lorsque la paille de lin non traitée ou traitée biologiquement est incubée en présence du foin.*

Mots-clés. *Production de gaz – Digestibilité – Paille – Foin – Mouton.*

I – Introduction

In tropical and sub-tropical developing countries, there is a gap between available and required animal feeds. Ruminants in these regions are often fed lignified forages and crop residues that are low in available energy and nitrogen. In these plants, a large proportion of structural carbohydrates is protected by lignin and thus not degraded by rumen bacteria (McSweeney *et al*., 1994; Sallam *et al*., 2007). In many ruminant production systems, cereal straws of low nutritive value are used as forages during part of the annual seasonal cycle. There is growing interest in the use of feeds with a high content of rapidly degradable fiber as supplements to ruminants consuming poor quality forage diets. Manyuchi *et al*. (1996) found that supplementation of poor quality natural pasture (veld) hay with napier and peanut hays increased the intake of veld hay. *In vitro* gas production measurements confirmed such positive associative effects between napier or peanut hays and veld hay (Wood and Manyuchi, 1997), and between rice straw and hay or mulberry leaves (Liu *et al*., 2002). Also, Nasser *et al*. (2006) concluded that positive associative effects on *in vitro* gas production occurred more consistently when untreated rice straw or biologically-treated rice straw with white rot fungi or mushroom was incubated in mixtures with hay than incubated alone. Li *et al*. (1998) found that body weight gain of Chinese Gelbvieh cattle was higher when animals were fed ammoniated wheat straw and corn stover given in mixture (50:50, w/w) than given separately. The *in vitro* gas production technique has been proposed to estimate ruminal degradation of feeds (Menke and Steingass, 1988; Getachew *et al.*, 1998), or digestible dry matter intake (DMI) and growth rate of cattle fed cereal straws (Blümmel *et al*., 1997a). The present study aimed at estimating *in vitro* gas production, energy value, organic matter digestibility and microbial protein production to investigate associative effects of untreated or biologically-treated linen straws with berseem hay.

II – Materials and methods

1. Feedstuffs and treatments

Berseem hay and untreated and biologically-treated linen straw were ground to pass a 1 mm sieve prior to chemical analyses and *in vitro* gas production measurements. The biological treatment with white rot fungi was performed according to Bassuny *et al*. (2003). Representative samples of untreated and biologically-treated linen straws and berseem hay were used for dry matter (DM), organic matter (OM), ether extract (EE), crude fiber (CF) and ash determination following the procedure of AOAC (1990). Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N $\times$ 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose and hemicelluloses were determined according to van Soest *et al*. (1991).

2. *In vitro* gas production, estimation of energy value, organic matter digestibility and microbial protein

The *in vitro* gas test method was performed according to Menke and Steingass (1988). Rumen content was collected before morning feeding from three ruminally fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. The rumen content was filtered through four layers of cheese-cloth and flushed with CO_2. The CO_2-flushed rumen fluid was added (1:2, v/v) to a buffered mineral solution (Onodera and Henderson, 1980), maintained in a water bath at 39℃. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. The gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 hours of incubation. Total gas values were corrected for blank incubation which contains only rumen fluid. Cumulative gas production Gas (t) at time t was fitted to the modified exponential model described by Dhanoa (1988). The energy value and organic matter digestibility (OMD) of forages were calculated from the gas produced according to Menke *et al*. (1979). Microbial protein production was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski (1986).

3. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM). Significant differences between individual means were identified using least significance difference (LSD) multiple range test (SAS, 2000).

III – Results and discussion

1. Chemical composition

Chemical composition of hay, untreated and biologically-treated linen straw is presented in Table 1. Large differences in chemical composition were evidenced between of H, LS and TLS. CP content varied from 3.61% for LS to 15.32% for H. The CP content of H was higher than those of LS and TLS.

Table 1. Chemical composition of the investigated feedstuffs

Items (%)	Hay (H)	Untreated linen straw (LS)	Treated linen straw (TLS)
Organic matter (% DM)	88.8	74.1	67.2
Crude protein (% DM)	15.3	3.6	9.3
Ether extract (% DM)	1.0	1.4	0.4
Ash (% DM)	11.20	25.95	32.79
Crude fiber (% DM)	29.8	54.3	23.2
NDF (% OM)	54.9	84.6	81.7
ADF (% OM)	38.3	71.1	66.0
ADL (% OM)	12.3	26.0	25.1

The treatment by fungi increased CP content of TLS compared to that of LS. It is well known that CP of forages is highly influenced by the biological treatments (Neijat and Gallagher, 1997; Nasser *et al*., 2006) and the chemical treatments (Liu *et al*., 2002). The OM content was lower for TLS than for H and LS. The NDF, ADF and cellulose content of feedstuffs ranged from 54.92 to 84.58, 38.31 to 71.14 and 26.04 to 45.14% OM, respectively. There was a reduction in the NDF, ADF, and cellulose content of the treated straw. These results are in agreement with findings of Neijat and Gallagher (1997) and Nasser *et al*. (2006). Ash content varied from 11.2% for H to 32.79% for TLS.

2. Gas production and estimated parameters

Data on gas production are given in Table 2. The cumulative volume of gas increased with increasing time of incubation (Fig. 1). Significant differences in gas production were evidenced between substrates for all the incubation times. Thus, gas productions were higher ($P < 0.01$) with H than with untreated and TLS. Total gas produced at 96 h of incubation (Fig. 1; Table 2) was highest for H ($P < 0.01$) and greater for H + LS and H + TLS ($P < 0.05$) than LS and TLS. When LS and TLS were mixed with hay, the total gas production and the rate of gas production increased, which are consistent with the results reported by Liu *et al*. (2002) and Nasser *et al*. (2006). Liu *et al*. (2002) showed that positive associative effects on *in vitro* gas production occurred more consistently when rice straw was incubated in mixtures with hay or mulberry leaves than when incubated in mixtures with chemically treated rice straw. Wood and Manyuchi (1997) observed statistically significant positive associative effects of veld hay and napier hay or peanut hay fermented in both N-rich and N-free media as assessed by gas production and DM disappearance.

The gas production from the insoluble fraction (b) was highest for H ($P < 0.01$) and greater for H + LS and H + TLS ($P < 0.05$) than LS and TLS. The gas production rate (c) of LS and TLS was significantly lower ($P < 0.05$) than those of H, H + LS and H + TLS. An increase in NDF and ADF resulted in the low gas production rate (c) of LS and TLS. This result is in agreement with findings of Kamalak (2006). Ndlovu and Nherera (1997) found that gas production rate (c) was negatively correlated with NDF and ADF. On the other hand, gas production and estimated parameters (c, a, b and a + b) were positively correlated with CP which is one of the limiting factors for microbial growth (Larbi *et al*., 1998; Kamalak, 2006).

Table 2. Cumulative gas production at different times of incubation and gas production parameters for individual feedstuffs and mixtures of untreated and treated linen straw with hay

Feedstuffs	Cumulative gas (ml) produced at				Parameters of gas production†		
	12 h	24 h	48 h	96 h	b (ml)	c (ml/h)	Lag (h)
H	26.73^{b}	35.69^{b}	49.58^{c}	49.58^{c}	50.79^{c}	0.070^{b}	0.340^{bc}
LS	6.99^{a}	10.01^{a}	14.01^{a}	14.01^{a}	14.37^{a}	0.044^{a}	1.387^{c}
TLS	4.16^{a}	8.32^{a}	10.32^{a}	10.42^{a}	10.23^{a}	0.046^{a}	2.069^{bc}
H+ LS	19.61^{b}	28.14^{b}	33.80^{b}	33.80^{b}	29.08^{b}	0.053^{ab}	1.353^{b}
H + TLS	20.40^{b}	26.06^{b}	32.06^{b}	32.06^{b}	25.37^{b}	0.056^{ab}	1.651^{a}

†b: theoretical maximum of gas production; c: gas production rate; L: lag time.
[a,b,c]Means within the same column with differing superscripts are significantly different.

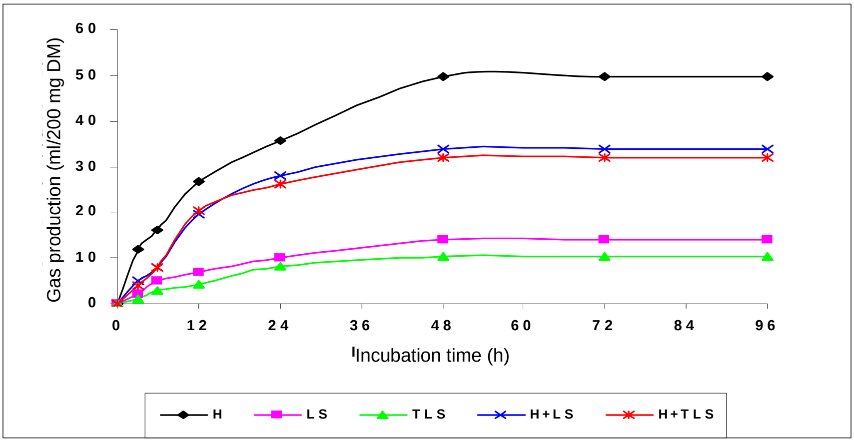

Fig. 1. Gas production profile for treated and untreated linen straw and there mixture with berseem hay (1:1) incubated *in vitro* for 96 h.

3. Energy contents and organic matter digestibility

The predicted metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), OMD and microbial protein (mg/kg DM) for individual feedstuffs and the different mixtures of untreated or biologically- treated linen straw with hay are presented in Table 3. Our data indicates that the ME and NE were highest ($P < 0.01$) for H and greater for H + LS and H + TLS ($P < 0.05$) than for LS and TLS. This result is in agreement with findings of Kamalak (2006). ME was positively correlated with CP protein which is one of the limiting factors for microbial growth (Larbi *et al*., 1998).

The OMD and microbial protein were higher ($P < 0.01$) for H, H + LS and H + TLS than for LS and TLS (Table 3). A close correlation exists between *in vitro* gas production and digestibility; the best correlation is achieved when the equation includes CP, crude fat and ash content (Menke and Steingass, 1988). *In vitro* gas production and *in vitro* apparent and true digestibility are highly correlated (Blümmel *et al*., 1997b). Gas production is an indirect measure of substrate degradation and is a good predictor for the production of volatile fatty acids, but it is not always positively related to microbial mass production. Blümmel *et al*. (1997b) suggested that there is an inverse relationship between *in vitro* gas production and microbial biomass yield.

Table 3. Predictions of metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD) and microbial protein synthesis prediction for individual feedstuffs and different mixtures of untreated and treated linen straw with hay

Items	ME (MJ/kg DM)	NE (MJ/kg DM)	OMD (%)	Microbial protein (g/kg DM)
Hay	7.94[c]	5.20[c]	54.34[c]	65.55[c]
LS	3.78[a]	2.56[a]	27.09[a]	32.68[a]
TLS	3.86[a]	2.52[a]	28.59[a]	34.49[a]
H + LS	6.57[b]	4.34[b]	45.37[b]	54.37[b]
H + TLS	6.45[b]	4.20[b]	45.02[b]	54.31[b]

[a,b,c]Means, within columns, with different superscript are significantly differ ($P < 0.05$).

IV – Conclusions

There are positive associative effects on *in vitro* gas production, which occurred more consistently when untreated and biological treated linen straw was incubated in mixtures with hay. We can improve the degradation of linen straw by mixing with high quality roughage as berseem hay without the need for biological treatments.

References

AOAC, 1990. *Official Methods of Analysis*, 13th edn. Washington DC, USA: Association of Official Analytical Chemists.

Bassuny S.M., Abdel-Aziz A.A., Ghanium A.Z. and Abdel-Aziz M.Y.S., 2003. Fibrous crop by-products as feed. 3. Effects of treatment rice bean straws by two kinds of fungus on chemical composition, cell wall constituents digestibility and rumen volatile fatty acid fractions of sheep. In: *Egyptian J. Nutrition and Feeds*, 6 (Special Issue). p. 913-924.

Blümmel M., Makkar H., Chisanga G., Mtimuni J. and Becker K., 1997a. The prediction of dry matter intake of temperate and tropical roughages from *in vitro* digestibility/gas production data, and the dry matter intake and *in vitro* digestibility of African roughages in relation to ruminant live weight. In: *Anim. Feed Sci. Technol*., 69. p. 131-141.

Blümmel M., Steingass H. and Becker K., 1997b. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and ^{15}N incorporation and its implications for the predictions of voluntary feed intake of roughages. In: *Br. J. Nutr*., 77. p. 911-921*.*

Czerkawski J.W., 1986. *An Introduction to Rumen Studies.* Oxford, New York: Pergamon Press.

Dhanoa M.S., 1988. On the analysis of the Dacron bag data for low degradability feeds. In: *Grass Forage Sci*., 43. p. 441-444.

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

Kamalak A., 2006. Determination of nutritive value of leaves of a native grown shrub, *Glycyrrhiza glabra* L. using *in vitro* and *in situ* measurements. In: *Small Ruminant Research*, 64. p. 268-278.

Larbi A., Smith J.W., Kurdi I.O., Adekunle I.O., Raji A.M. and Ladipo D.O., 1998. Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in humid tropics. In: *Anim. Feed Sci. Technol.*, 72. p. 81-96.

Li Y., Gu C., Cao Y., Liu R. and Li J., 1998. Relationship between intake and degradability of forages and their associative effect. In: *Proc. 3rd National Symposium on Feed Sciences and Animal Nutrition*, Chengdu (Sichuan, China), 28-31 October 1998. Beijing, China: China Society of Animal Nutrition. p. 153.

Liu J.X., Susenbeth A. and Sudekum K.H., 2002. *In vitro* gas production measurements to evaluate

interactions between untreated and chemically treated rice straw, grass hay, and mulberry leaves. In: *J. Anim. Sci.*, 80. p. 517-524.
Manyuchi B., Hovell F., Ndlovu L., Topps J. and Tigere A., 1996. Feeding napier hay as supplement to sheep given poor quality mature hay: Effects of level of napier hay supplement and inclusion of urea in the basal diet on intake and digestibility. In: *Anim. Feed Sci. Technol.*, 63. p. 123-135.
McSweeney C.S., Dulieu A., Katayama Y. and Lowry J.B., 1994. Solubilization of lignin by the ruminal anaerobic fungus *Neocallimastix patriciarium*. In: *Appl. Environ. Microbiol.*, 60. p. 2985-2989.
Menke K.H., Raab L., Salewski A., Steingass H., Fritz D. and Schneider W., 1979. The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. In: *J. Agric. Sci.* (*Cambridge*), 92. p. 217-222.
Menke K.H. and Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. In: *Anim. Res. Dev.*, 28. p. 7-55.
Nasser M.E.A., Sallam S.M.A., El-Waziry A.M., Hagino A., Katoh K. and Obara Y., 2006. *In vitro* gas production measurements and estimated energy value and microbial protein to investigate associative effects of untreated or biological treated rice straws with berseem hay. In: *2nd International Scientific Congress for Environment*, South Valley University (Qena, Egypt), 28-30 March 2006.
Ndlovu L.R. and Nherera F.V., 1997. Chemical composition and relationship to *in vitro* gas production of Zimbab-wean browsable indigenous tree species. In: *Anim. Feed Sci. Technol.*, 69. p. 121-129.
Neijat M.S. and Gallagher J.R., 1997. Chemical composition and nutritive value of pea straw subjected to fungal fermentation. In: *Trop. Anim. Health Prod*., 4. p. 216-218.
Onodera R. and Henderson C., 1980. Growth factors of bacterial origin for the culture of the rumen oligotrich protozoon, *Entodinium caudatum*. In: *J. Appl. Bacteriol.*, 48. p. 125-134.
Sallam S.M.A., Nasser M.E.A., El-Waziry A.M., Bueno I.C.S. and Abdalla A.L., 2007. Use of an *in vitro* rumen gas production technique to evaluate some ruminant feedstuffs. In: *J. Applied Sciences Research*, 3(1). p. 34-41.
SAS, 2000. *SAS Users Guide Statistical Analyses Systems Institute*. Cary, USA: Statistical Analysis Systems Institute.
van Soest P.J., Robertson J.B. and Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. In: *J. Dairy Sci.*, 74. p. 3583-3597.
Wood C.D. and Manyuchi B., 1997. Use of an *in vitro* gas production method to investigate interactions between veld hay and napier hay or groundnut hay supplements. In: *Anim. Feed Sci. Technol*., 67. p. 265-278.