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Diets with Different Forage/Concentrate Ratios for the Mediterranean Italian Buffalo: *In vivo* and *In vitro* Digestibility

Zicarelli Fabio*, Serena Calabrò, Vincenzo Piccolo, Simona D'Urso, Raffaella Tudisco
Fulvia Bovera, Monica I. Cutrignelli and Federico Infascelli
University of Napoli Federico II, Department of Scienze Zootecniche e Ispezione degli alimenti
via F. Delpino, 1-80137 Naples, Italy

ABSTRACT : *In vivo* and *in vitro* digestibility of 6 diets with a forage to concentrate ratio (F/C) ranging from 100 to 50:50 (diet 1: all hay, diet 2: 90:10, diet 3: 80:20, diet 4: 70:30, diet 5: 60:40, diet 6: 50:50) were investigated using 6 buffaloes in a 6×6 Latin square design. For the *in vivo* trial, the individual faeces of buffaloes were collected 3 times per day for 7 days. Individual pooled faeces and samples of each diet were analysed for chemical composition and insoluble acid ash (AIA) contents in order to estimate the coefficient of apparent digestibility (ADC). On the last day of the *in vivo* trial a sample of faeces was collected from each animal and used as inoculum for the *in vitro* test, using the gas production technique (IVGPT). The *in vivo* organic matter digestibility (ADC) rose as the percentage of concentrate increased up to the 70:30 (F/C) diet (67.01, 73.03, 78.06 and 79.05, respectively for diets 1, 2, 3 and 4); the other two diets (60:40 and 50:50 F/C) unexpectedly did not follow this trend (75.11 and 79.06, respectively for diet 5 and 6). However, these data agree with the results of the *in vitro* trial. The ADC was positively correlated with the dOM ($p<0.001$), but not with the gas production at different times; cumulative gas production recorded at the end of incubation (OMCV) showed an irregular trend and was not closely correlated to degraded OM. Estimation of *in vivo* digestibility from *in vitro* fermentation data was acceptable, despite leaving room for improvement. (**Key Words :** *In vitro* Gas Production Technique, *In vivo* Digestibility, Faeces, Buffalo)

INTRODUCTION

In the last ten years, the importance of the Mediterranean Italian Buffalo (*Bubalus bubalis*, L.) in the Campania region of southern Italy has increased due to rising demand for buffalo mozzarella cheese, which has been listed by the EU as Protected Designation of Origin (PDO). Several researchers from different disciplines are currently involved in studying the physiological traits of this species. In order to contribute to the knowledge of buffalo nutrition, it is useful to study the estimation of organic matter (OM) digestibility which is a basic step in evaluating the net energy content of feeds.

OM digestibility of feeds can be estimated *in vivo* using the ingesta-excreta balance, by the marker method (Van Soest, 1996; McDonald et al., 2002), the *in situ* method (Mehrez and Ørskov, 1977) or by *in vitro* techniques (Tilley and Terry, 1963; Menke et al., 1979; Aufere, 1982; Kopecny et al., 1989; Pell and Schofield, 1993; Theodorou et al., 1994; Bovera et al., 2006). In recent years, the *in vitro* gas production technique (IVGPT) has been used to evaluate the OM digestibility of forages; this method, by which feedstuffs are incubated with a rumen fluid inoculum, can also give information on the degradation rate of feedstuffs.

Due to the easy employment of this technique, several researchers have compared *in vivo* digestibility and feed intake with the results of the IVGPT. Data from 400 tests of *in vivo* digestibility trials and the respective IVGPT were used to construct equations to provide *in vivo* digestibility from gas production (Menke and Steingass, 1988). Blümmel and Ørskov (1993) studied the correlations between the total gas production of 10 straws and the dry matter intake, OM digestibility and live weight gain. Total gas production was correlated with intake (0.88), digestible dry matter intake (0.93) and growth rate (0.95) in a multiple regression model.

Calabrò et al. (1997), using diets at different forage/concentrate ratios in the buffalo, found a significant ($p<0.001$) correlation between *in vivo* DM digestibility and the volume of gas produced after 48 h of incubation (R^2 = 0.996) and the potential gas production (R^2 = 0.990).

* Corresponding Author: Fabio Zicarelli. Tel: +39-081-2536284, Fax: +39-081-292981, E-mail: fabiozicarelli@gmail.com
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Table 1. Ingredients and chemical composition of the diets

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredients (% as feed)						
Oat hay	100	90.0	80.0	70.0	60.0	50.0
Concentrates*	-	10.0	20.0	30.0	40.0	50.0
Chemical composition						
DM (%)	90.21	90.43	89.72	89.33	89.65	89.62
Ash (% DM)	9.62	8.80	8.40	7.20	6.80	8.20
CP (% DM)	11.51	11.72	11.62	11.62	11.32	11.42
NDF (% DM)	63.93	59.64	56.04	52.51	47.92	44.45
ADF (% DM)	43.62	40.51	36.02	31.22	28.21	24.07
ADL (% DM)	7.31	6.80	6.20	5.60	5.00	4.30
NFC (% DM)	12.03	16.92	21.03	25.75	31.03	33.01

* Concentrate: 36% wheat bran+64% maize meal.
DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin; NFC (non-fiber carbohydrates) = 100-(NDF+CP+Ash+Crude fat).

Although rumen fluid for the IVGPT is usually collected from rumen-fistulated animals, the method is costly both financially and in terms of animal welfare, hence the interest in an alternative microbial source. Several researchers (Manyuchi et al., 1991; Gonçalves and Borba, 1996; Jones and Barnes, 1996; Omed et al., 1998) have shown that ruminant faeces can be used for *in vitro* trials. Mauricio et al. (2001) concluded that faeces have potential as an alternative inoculum to rumen fluid for *in vitro* gas production techniques, but methods of overcoming the longer lag phase with faeces require further research.

The aim of the present paper is to compare the *in vivo* OM digestibility and the *in vitro* fermentation characteristics of six diets with different forage/concentrate ratios in the Mediterranean Italian buffalo. For the *in vivo* trial acid insoluble ash was used as marker and for the IVGPT the faeces of the animals were used as inoculum. The correlations between *in vivo* OM digestibility and the IVGPT parameters were also studied.

MATERIAL AND METHODS

The trial was carried out using six Mediterranean Italian buffalo heifers (*Bubalus bubalis* L.), 22 months old and 400 kg average live weight, in a 6×6 Latin square design. The animals were housed in individual boxes in order to check feed intake and refusals. Animals were fed six iso-protein diets (N×6.25 = 115 g/kg DM) with different forage:concentrate ratios: Diet 1 = 100 (all hay); Diet 2 = 90:10; Diet 3 = 80:20; Diet 4 = 70:30; Diet 5 = 60:40 and Diet 6 = 50:50, oat hay being the forage for all the diets. Concentrate comprised maize meal (64%) and wheat bran (36%) with an appropiate mineral-vitamin supplementation. The ingredients and chemical composition of the diets are reported in Table 1.

In vivo digestibility

The diets were offered twice daily, at 08:00 am and 04:00 pm, as total mixed ration (TMR). Each period was divided into two sub-periods: the first (14 days) for adaptation to the new diet and to evaluate the voluntary feed intake, and the second (7 days) for faeces collection (3 times/day). In the second sub-period, in order to avoid refusals, each animal was fed in an amount equal to the voluntary feed intake.

Samples of the TMR were collected daily, dried at 62°C for 48 h, ground through a 1mm screen (Braebender Wiley mill, Braebender OHG, Duisburg, Germany) and analysed for dry matter (DM), crude protein (CP) and ash as suggested by the AOAC (2000) procedures (ID members: 930.04, 930.05, 977.02 and 930.10, respectively). Neutral detergent fibre (NDF) was determined by boiling for 1 h a 0.5 g sample in 100 ml of neutral detergent plus 50 μl of heat stable α-amylase (ANKOM Technology) and 0.5 g of sodium sulphite (Van Soest et al., 1991). Acid detergent fibre (ADF) and lignin (ADL) were determined according to Goering and Van Soest (1970).

The faecal samples were collected for each buffalo directly from the rectum as suggested by Rode et al. (1999). They were then dried at 65°C, ground through a 1-mm screen (Brabender Wiley mill, Braebender OHG, Duisburg, Germany) and analysed for dry matter (DM) and ash as suggested by the AOAC (2000) procedures (ID members: 930.04). The acid insoluble ashes (AIA) were determined as suggested by Van Keulen and Young (1977) and the coefficients of apparent digestibility (ADC) of each diet were calculated.

In vitro trial

A sample of faeces was collected on the last day of each *in vivo* trial (at 8.00 am), from the rectum of each buffalo, put in pre-warmed thermos and rapidly transported to the laboratory. About 50 g of faeces were mixed with 100 ml of anaerobic buffer (Medium D; Theodorou, 1993); the whole mixture was filtered through various layers of gauze and then diluted 1:1 (Mauricio et al., 2001) with the buffer,

Table 2. Apparent digestibility coefficients of the organic matter in the diets

	ADC (%)
Diet 1	67.01^{D}
Diet 2	73.03^{C}
Diet 3	78.06^{ABa}
Diet 4	79.05^{A}
Diet 5	75.11^{BCb}
Diet 6	79.06^{A}
SEM	1.09

ADC: Apparent digestibility coefficients.
^{A,B,C,D} Values with different letters are significantly different ($p<0.01$).
^{a,b} Values with different letters are significantly different ($p<0.05$).
SEM: Standard error mean.

ultimately obtaining six inocula (1 per buffalo). The various steps were carried out at 39°C and under insufflations of CO_2 in order to maintain anaerobic conditions.

Around 1 g of sample per diet was incubated as substrate in 3 replications at 39°C in 100 ml culture flasks containing 74 ml of anaerobic medium and 5 ml of inoculum. The fermentation was carried out for 144 h. At pre-established times (at 2-24 intervals) the gas produced was measured for each flask using a pressure transducer described by Theodorou et al. (1994). At the end of incubation an aliquot (5 ml) of the liquid flask content was used to determine the pH (Alessandrini Instrument glass electrode; mod. JENWAY 3030) and the volatile fatty acid (VFA) including acetate, propionate and butyrate. The content of each flask was filtered using pre-weighed porous septum crucibles (Schott Duran, porosity 2) and the residual OM was determined by drying at 103°C and burning at 550°C. Degraded OM (dOM, %) was calculated by the difference between incubated and residual OM, corrected for the blank, consisting of 4 flasks containing only the inoculum.

For VFA analysis the liquid sample was centrifuged twice at 12,000 g for 10 min at 4°C. One ml of supernatant was added to 1 ml of oxalic acid 0.06 M. Volatile fatty acids were measured by gaschromatography (Thermo Quest mod. 8000^{top}, FUSED SILICA capillary column 30 m×0.25 mm× 0.25 mm film thickness) according Calabrò et al. (2006) including acetate, propionate and butyrate as external standards. The area of each VFA response was compared with the external standard.

Statistical analysis

For each inoculum, the cumulative gas volumes obtained at each incubation time (average of the three bottles), as ml/g of incubated organic matter (ml/g OM), were elaborated using a multiphasic model suggested by Schofield et al. (1994):

$$V = V_F (1+\exp (2+4 S (\lambda-t)))^{-1} \quad \text{Equation 1}$$

where V_t is the gas volume at time t; V_F is the maximum volume at time $t = \infty$, S is a rate constant called the specific rate (S = maximum rate/maximum volume), and λ is an integration constant equivalent to a lag term. The dual-pool version of Equation 1 would contain two terms, each with its own values for V_F and S, but with the same value for λ. The NONLIN package (Sherrod, 1995) was used to fit the data to this equation.

The diets were compared for the *in vivo* (the apparent coefficient digestibility, ADC) and *in vitro* data, i.e. model parameters, dOM, OMCV (gas production at 144 h of incubated OM), VFA and pH, with the following model:

$$y_{ij} = \mu+D_i+\varepsilon_{ij}$$

where y is the single data, μ is the mean, D is the diet effect (1-6) and ε is the error. The t-test was used to assess statistically the *in vitro* and *in vivo* characteristics.

In order to evaluate the relationships between the *in vivo* and *in vitro* results, the correlation between the ADC and the IVGPT parameters (dOM and the real gas recorded after 24, 48, 72, 96, 120 and 144 hours of incubation (G24, G48, G72, G96, G120 and G144, respectively)) was studied using the CORR procedure of SAS (2000).

RESULTS

***In vivo* digestibility**

The apparent digestibility coefficients (ADC) of the organic matter in the six diets are reported in Table 2. According to the concentrate content of the diets, the ADC values increased up to diet 4 (diets 1, 2 and 3 differed for $p<0.01$); diet 5 showed significantly ($p<0.01$) lower values than diets 3 and 4, while diet 6 was superimposable on these last two diets.

***In vitro* fermentation characteristics**

The parameters of the *in vitro* fermentation (dOM, OMCV, acetate, propionate, butyrate, total VFA), acetate:propionate ratio (A:P) and pH) are shown in Table 3. OM degradability increased as the concentrate of diets increased in the first 4 diets: diet 1 was less degradable than diets 2 and 3 ($p<0.05$) and 4 ($p<0.01$). As observed *in vivo*, despite its higher concentrate content diet 5 showed lower OM digestibility than the previous diets, even if the differences were not statistically significant. OM digestibility also increased from diet 5 to diet 6. On increasing the concentrate in the diet, the pH values decrease.

DISCUSSION

The cumulative gas production recorded at the end of

Table 3. *In vitro* fermentation characteristic of the diets

	dOM (%)	OMCV (ml/g)	Acetate (mmol/g)	Propionate (mmol/g)	Butyrate (mmol/g)	VFA (mmol/g)	A:P	pH
Diet 1	61.06^{Bb}	168.81^{D}	20.57^{BC}	7.05^{Cb}	2.15^{b}	30.56^{Bb}	3.13^{a}	6.69^{Aa}
Diet 2	67.54^{a}	196.02^{C}	22.00^{B}	7.70^{Cb}	1.97^{b}	32.08^{Bab}	2.84^{ab}	6.62^{A}
Diet 3	68.63^{a}	179.54^{CD}	25.80^{bc}	9.80^{Cb}	2.55^{ab}	38.47^{Bab}	2.60^{ab}	6.52^{ab}
Diet 4	69.69^{A}	192.32^{C}	26.70^{abc}	9.38^{Cb}	3.57^{ab}	45.11^{ABa}	2.74^{ab}	6.43^{bc}
Diet 5	64.80^{ab}	218.72^{B}	28.09^{ab}	12.05^{b}	4.01^{a}	46.12^{ABa}	2.63^{ab}	6.28^{Bc}
Diet 6	68.23^{a}	239.81^{A}	33.41^{Aa}	20.10^{Aa}	3.80^{a}	58.60^{Aa}	1.66^{b}	6.23^{Bc}
SEM	2.88	7.97	3.46	3.04	0.78	6.92	0.68	0.11

Diet 1: all hay; Diet 2: F/C = 90/10; Diet 3: F/C = 80:20; Diet 4: F/C = 70/30; Diet 5: F/C = 60:40; Diet 6: F/C = 50/50.
dOM = Organic matter degradability (% of incubated); OMCV = Cumulative gas volume of incubated OM.
VFA = Total volatile fatty acids; A:P = Acetate-propionate ratio.
In the same column values with different letters are significantly different (^{a,b} $p<0.05$ and ^{A,B,C,D} $p<0.01$).
SEM: standard error mean.

Table 4. Fermentation parameters obtained by monophasic and biphasic models

	V_f (ml)	V_s (ml)	V_f(%)	$S_f(h^{-1})$	$S_s(h^{-1})$	F	V (ml)	S (h^{-1})	F
	Biphasic model						Monophasic model		
Diet 1	36.33^{Cc}	139.01^{Aa}	20.69^{C}	0.0567^{bc}	0.0094^{b}	5,055	173.63^{D}	0.0082^{Bb}	649
Diet 2	65.01^{BCbc}	132.03^{Aa}	33.38^{BCb}	0.0493^{Bc}	0.0117^{ab}	3,821	192.55^{BCc}	0.0119^{b}	765
Diet 3	81.67^{ABC}	98.67^{b}	44.95^{AB}	0.0513^{c}	0.0117^{ab}	1,189	168.53^{D}	0.0110^{b}	203
Diet 4	116.33^{A}	79.47^{B}	59.30^{A}	0.0457^{Bc}	0.0100^{ab}	889	172.81^{D}	0.0170^{Aa}	136
Diet 5	104.33^{ABa}	109.02^{ABab}	48.70^{AB}	0.0863^{Aa}	0.0120^{a}	1,419	202.75^{ABCb}	0.0167^{Aa}	167
Diet 6	124.9^{A}	111.01^{ABab}	52.81^{ABa}	0.0770^{ab}	0.0106^{ab}	1,092	215.19^{Aa}	0.0183^{Aa}	120
SEM	16.49	15.50	7.33	0.0125	1.13×10^{-3}	-	4.53	2.6×10^{-3}	-

Diet 1: all hay; Diet 2: F/C = 90/10; Diet 3: F/C = 80:20; Diet 4: F/C = 70/30; Diet 5: F/C = 60:40; Diet 6: F/C = 50/50.
V_f: potential gas *pool* of the fast fraction in the biphasic model; V_s: potential gas *pool* of the slow fraction in the biphasic model;
V_f(%): $(V_f/(V_f+Vs))\times100$; S_f: relative rate of the fast fraction; S_s: relative rate of the slow fraction;
V: potential gas production in the monophasic model; S: relative rate of gas production.
Values with different letters (^{a,b,c} and ^{A,B,C,D}) in the same column are significantly different for $p<0.05$ and $p<0.01$, respectively.
SEM: standard error mean.

incubation (OMCV) showed an irregular trend and was not closely correlated to degraded OM, in contrast with the results of other authors (Blümmel and Ørskov, 1993; Calabrò et al., 1997). However, it must be underlined that not all the degraded organic matter is fermented, thereby producing gas. Williams (2000) indicates that the fate of the degraded OM is double: gas and volatile fatty acids production and microbial biomass production.

Generally, the VFA concentration agrees with the final gas production. Indeed, there was a close relationship between VFA and gas production during the fermentation process in rumen and caecum. The fermentation of the carbohydrates can be simplified by considering the glucose molecule as follows (Van Soest, 1996):

Glucose = 2 Acetate+CO_2+CH_4

Glucose = 1 Butyrate+2CO_2

Glucose = 2 Propionate.

The gas from the reaction between the VFA and HCO_3^- /CO_3^- buffer has to be added to the gas obtained from fermentation. The association between gas volume and VFA production is clear. To underline these considerations, a correlation coefficient (r) equal to 0.9088 ($p<0.01$) between the two parameters was found.

Organic matter digestibility

As reported by others (Mould et al., 1983; Hoover, 1986; Sniffen and Robinson, 1987; Huhtanen and Jaakkola, 1993), the forage:concentrate ratio in the diet influences *in vivo* structural carbohydrate digestibility. As the concentration of non-fibrous carbohydrates (mainly starch and sugars) increases, fibre digestion decreases, due to the lower rumen pH which alters the activity of the cellulolytic bacteria.

In the present experiment, diet 5 (F:C = 60:40) showed a decrease in OM digestibility, both *in vivo* and *in vitro*, compared to the other diets, probably due to the lower fibre digestion. At this result surely contributed the lower activity of the cellulolytic bacteria evidenced by the pH value (6.28), close to the threshold value (6.20) suggested for cellulolytic bacteria activity (Mould et al., 1983; Grant and Mertens, 1991; Huhtanen et al., 2006).

OM digestibility increases again in diet 6 (F:C = 50:50). The loss in digestibility of the structural carbohydrates may well be compensated by the higher contribution to the

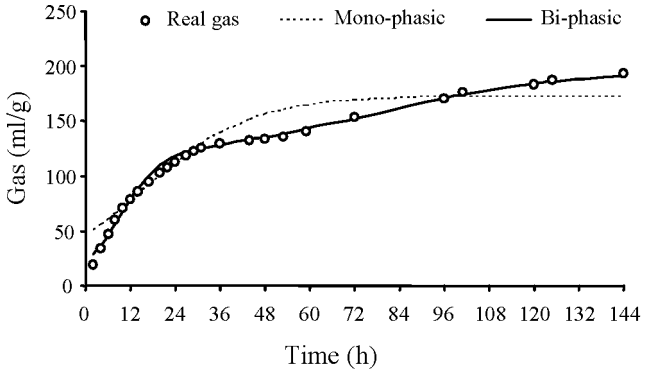

Figure 1. Typical gas production trend for Diet 4 (F/C = 70:30) using monophasic and biphasic models.

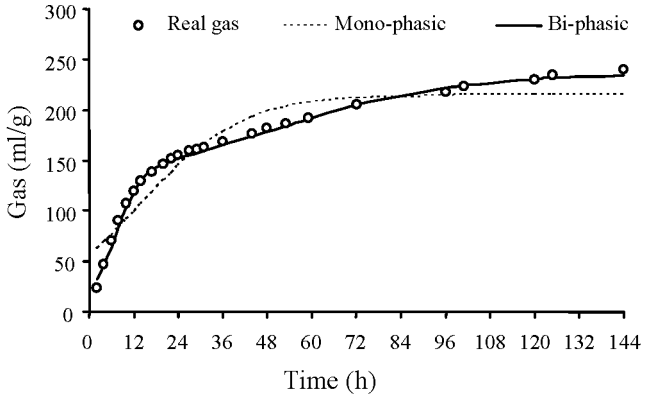

Figure 2. Typical gas production trend for Diet 6 (F/C = 50:50) using monophasic and biphasic models.

degraded OM of the non-fibre carbohydrates.

Fermentation kinetics and gas production

In our previous IVGPT experiments, with rumen fluid as inoculum (Calabrò et al., 2001 and 2004), the multiphasic model proposed by Groot et al. (1996) always gave a good fit to the experimental data; instead in the present trial with faecal inoculum, this model did not fit as well. Good results were obtained using the multiphasic model proposed by Schofield et al. (1994) which includes a lag phase. Figure 1 shows a typical gas production curve using monophasic and biphasic models.

In Table 4 the parameters of the curves obtained using a monophasic or biphasic model are shown. The biphasic showed better fitted the values of the cumulative gas production recorded, due to the higher number of estimated parameters as indicated by the higher *F* values: for the biphasic model ranging from 5,055 to 889 and for the monophasic model ranging from 765 to 120. Moreover, as reported by Doane et al. (1997a) the t values were also taken into account: for each parameter it was similar or better using the biphasic model. Consequently, only the results obtained using the biphasic model will be discussed below.

The biphasic model involves gas production with two different rates, higher (S_f) and lower (S_s), clearly distinguishable, associated to two different pools of gas V_f and V_s respectively. The two gas pools may be due to the fermentation of two different substrates, or two different microbial populations or probably, to the combination of both factors as hypothesized by some authors (Schofield et al., 1994; Schofield and Pell, 1995; Cone et al., 1996).

In order to illustrate the comparison among the diets, the gas volume associated to the most rapidly fermentable fraction is also reported (Table 4) as a percent of the total volume (V_f, %). Excluding diet 5, the gas volume (V_f) associated to the higher rate increased according to the concentrate content of diet (Table 4). Diets 1 and 2 were significantly different from diets 4, 5 and 6 ($p<0.01$). Diet 3 showed a significant difference ($p<0.05$) from diet 1. The gas volume trend of the lower rate is less clear, as it decreased with the concentrate up to the first four diets and then increased. The gas proportion trend of the higher rate (V_f, %) is related more closely to the chemical composition of the diets. Indeed, on average, V_f (%) increased by 12.9 units for each 10-point increase in concentrates up to diet 4; diets 5 and 6 then recorded lower values than diet 4. The relative rate of the pool V_f (S_f) was higher in the two diets with a lower forage/concentrate ratio, while that of pool V_s (S_s) was lower for diet 1, significantly differing only from the diet 5 ($p<0.05$).

In general, the gas proportion of the rapidly fermentable fraction (V_f, %) agrees with that reported by Schofield and Pell (1995) and Doane et al. (1997a) for single forages. The gas fraction associated to the faster pool decreased as fibre content in the diet increased.

As both gas pools were estimated by a mathematical model, they are not clearly connectable to a specific chemical entity. As a rule, as supposed by Doane et al. (1997a), it is possible that to both pools different Neutral Detergent Soluble (difference in gas produced between the unfractioned whole forage and its respective NDF) and NDF fractions contributed.

For all diets, gas production at 48 h of incubation (Table 5) was lower, proportionally to the weight of substrate, than that reported for forages (Schofield and Pell, 1995; Doane et al., 1997 a and b) and for diets (Calabrò, 1999). Probably the different findings were also due to the inoculum micropopulation which comes mainly from the caecum where it is adapted to carbohydrates and nitrogen with low fermentability and degradability, respectively. Hence it needs to conform to the new substrates. For sure, the peculiarity of the inoculum contributed to the slower rate of both pools, considering normal that obtained by Schofield et al. (1994). The key difference in gas production rates between rumen fluid and faeces as sources of micro-organisms can be explained by the different populations of

Table 5. *In vivo* digestibility and *in vitro* degradability of organic matter and gas production recorded at different times

	ADC (%)	dOM (%)	G 24 (ml/g)	G 48 (ml/g)	G 72 (ml/g)	G 96 (ml/g)	G 120 (ml/g)	G 144 (ml/g)
Diet 1	67.01^{b}	61.06^{Bb}	56.04^{Cd}	80.95^{C}	111.43^{Cc}	135.39^{Cc}	156.75^{Cc}	169.81^{C}
Diet 2	73.03^{C}	67.54^{a}	76.42^{BCcd}	112.74^{BC}	153.62^{BCb}	172.70^{BCb}	187.42^{BCab}	196.04^{BC}
Diet 3	78.06^{Aba}	68.63^{a}	89.31^{BCbc}	112.13^{BC}	138.21^{C}	154.22^{C}	168.37^{C}	179.46^{C}
Diet 4	79.05^{A}	69.69^{A}	115.13^{ABb}	136.65^{ABb}	154.45^{BCb}	169.94^{BCb}	182.77^{BCb}	192.26^{BCb}
Diet 5	75.11^{BCb}	64.80^{ab}	138.71^{A}	169.21^{A}	191.16^{ABa}	201.12^{ABa}	210.43^{ABa}	218.69^{ABa}
Diet 6	79.06^{A}	68.23^{a}	153.09^{Aa}	181.31^{Aa}	203.72^{A}	215.58^{A}	227.75^{A}	239.93^{A}
SEM	1.09	2.88	14.00	16.24	15.64	13.81	11.80	10.57

Diet 1: all hay; Diet 2: F/C = 90/10; Diet 3: F/C = 80:20; Diet 4: F/C = 70/30; Diet 5: F/C = 60:40; Diet 6: F/C = 50/50.
ADC = Apparent digestibility coefficients of the organic matter. dOM = *in vitro* degradability of the organic matter.
G 24, G 48, G 72, G 96, G 120 e G 144: gas production of incubated OM recorded at 24, 48, 72, 96, 120 and 144 h.
Values with different letters ($^{a, b, c, d}$ and $^{A, B, C, D}$) in the same column are significantly different for $p<0.05$ and $p<0.01$, respectively.
SEM: standard error mean.

Table 6. Estimation equations of *in vivo* organic matter digestibility from *in vitro* fermentation parameters

	Eq. No.	Intercept	b_1	b_2	R^2	RSD
y = ADC	1	-7.37	+1.26 dOM	-	0.695	2.90
y = ADC	2	-4.77	+1.40 dOM	-0.06 G120	0.790	2.48

ADC = Apparent digestibility coefficients of the organic matter (%).
dOM = *in vitro* degradability of the organic matter (%).
G 120 = Gas production recorded at 120 h of incubation (ml/g).
RSD = Residual standard deviation.

micro-organisms present as suggested by Mauricio et al. (2001).

Correlations between *in vivo* OM digestibility and *in vitro* fermentation parameters

In Table 5 *in vivo* digestibility, *in vitro* degradability of the organic matter and cumulative gas production at various times chosen according to the consulted bibliography (Menke et al., 1979; Blümmel and Ørskov, 1993; Macheboeuf et al., 1997; Khazaal et al., 1995; Calabrò, 1999) are shown. The *in vitro* organic matter degradability (dOM) was always lower than *in vivo* digestibility (ADC), while the two trends were almost superimposable.

Cumulative gas productions after 144 h of fermentation (G144) do not always agree with the dOM. Diet 5, with a low degradability (64.8%), showed high final gas production (218.7 ml); vice versa diet 3 (high dOM 68.63 and low gas production 179.5 ml). The ADC is positively related with dOM ($p<0.001$), but not with gas production at every time. Usually both the dOM and ADC are related to gas production, but in our case the results may well have been influenced by the faecal inoculum.

In Table 6 the results of the regression study of the ADC on the parameters of *in vitro* fermentation are reported. Equation (N.1) for estimating the ADC as a function of the dOM was obtained ($p<0.05$) with R^2 equal to 0.694 and RSD 2.90, which leaves considerable room for improvement.

Using the step-wise procedure, the equation also includes the gas produced at 120 h (Eq. N.2) improving the R^2 (0.790 vs. 0.694; $p<0.05$) and RSD (2.47 vs. 2.90) values.

CONCLUSIONS

This work confirmed the IVGPT as a straightforward, reliable method to study the characteristics of ruminant diets. Moreover, our data indicated the importance of a suitable mathematical model to fit gas production values and properly describe the fermentation kinetics of the substrates. In addition, estimation of the ADC from *in vitro* fermentation data was attained, albeit leaving room for improvement.

The results of this experiment showed that faecal matter has potential as an alternative inoculum to rumen fluid for the IVGPT, in order to avoid using fistulated animals, and appears an eminently practical method. However, for the reliable applicability of faeces as inoculum for the gas production technique, further work is required to correct these profiles according to their corresponding rumen fluid profiles.

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