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# Comparison of Rabbit Caecal Content and Rabbit Hard Faeces as Source of Inoculum for the *In vitro* Gas Production Technique

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**ABSTRACT :** In order to find an alternative source of inoculum to caecal content for studying the fermentation activity of rabbit hindgut, caecal content and faeces of 25 hybrid Hyla rabbits were used as inocula for an *in vitro* gas production trial. About 1 g of three substrates (dehydrated alfalfa meal, dehydrated beet pulp, barley) was weighed, in quadruplicate per inoculum, in 120 ml bottles; 75 ml of anaerobic medium and 4 ml of reducing solution were added and bottles were placed at 39°C. Caecal content and faeces were diluted respectively 1:2 (CI) and 1:8 (FI) with anaerobic medium and were introduced in the respective bottles (10 ml). Gas production was recorded 20 times at 2-24 h intervals throughout fermentation (96 h). The fermentation characteristics (i.e. degraded organic matter, OMd; potential gas production, A; fermentation rate, Rmax; time at which it is reached, Tmax; pH, volatile fatty acid, VFA) were studied by inoculum and feedstuffs. The feedstuffs, according to their chemical composition, showed very different fermentation characteristics. In particular, OMd, A and Rmax allowed feedstuff classification as follows: barley>beet pulp>alfalfa. The inocula differ ($p<0.05$) in Tmax, were higher for CI (15.53 vs. 11.96 h) and in VFA production. In particular, CI produced higher levels of acetate (38.9 vs. 33.4 mM/g OM incubated, $p<0.01$) and isobutyrate (0.72 vs. 0.42, $p<0.01$) but less propionate (7.1 vs. 10.3, $p<0.01$) and butyrate (11.3 vs. 14.0, $p<0.01$). However, the trend of gas production, similar for the inocula according to the fermented substrate, and the good regression equation to estimate some caecal fermentation parameters from faeces suggest that, after standardisation, the faeces could be used as an alternative inoculum for gas tests in rabbit. (**Key Words :** *In vitro* Gas Production Technique, Faeces, Caecal Content, Rabbit)

## INTRODUCTION

Since the 1990s there has been increasing interest in the *in vitro* technique that measures gas production both to study the kinetics of rumen fermentations (Blümmel et al., 1997; Getachew et al., 1998; Calabrò et al., 2002) and to estimate the *in vivo* digestibility of ruminant feeds (Menke and Steingass, 1988; Blümmel et al., 1993). On the basis of the valid results obtained, the relatively straightforward low-cost trials and the development of automated systems (Pell and Schofield, 1993; Cone et al., 1996; Davies et al., 2000) able to record gas production continuously during fermentation, the *in vitro* gas production technique (IVGPT) was recently developed to characterize feed ingredients also in single-stomached animals (Williams et al., 1997). In rabbits the IVGPT was used in order to study the fermentative characteristics of feedstuffs and diets (Calabrò et al., 1999; Stanco et al., 2003). Recently, due to the increasing interest on the weaning period (Nizza et al., 2004; Piccolo et al., 2005) the IVGPT was also used to study the changes in microbial activity of caecum microflora around weaning (Gazaneo et al., 2003).

The IVGPT is based on the fact that the anaerobic digestion of carbohydrates by rumen or caecal micro-organisms produces gas ($CO_2$, $CH_4$ and traces of $H_2$) and volatile fatty acids (acetate, propionate, butyrate); gas production can be measured to estimate the rate and extent of feed degradation. The IVGPT needs feeds (substrates), an anaerobic medium and a representative sample of the micro-organism population present in the rumen or caecum (inoculum).

While in ruminants it is possible to approach the rumen directly by surgery, in rabbits the sampling of caecal content to use as inoculum for gas tests is tied to animal slaughter. However, in both cases, there are a number of ethical considerations regarding these techniques with respect to animal welfare. It is thus necessary and urgent to find an alternative source of inoculum for IVGPT. A number of studies (El Shaer et al., 1987; Akhter et al., 1995;

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**Table 1.** Chemical composition of feedstuffs (% on dry matter basis)

| | CP | CF | EE | Ash | NDF | ADF | ADL |
|---|---|---|---|---|---|---|---|
| DAM | 19.35 | 29.16 | 3.04 | 4.40 | 47.32 | 35.21 | 10.00 |
| DBP | 9.18 | 20.18 | 0.67 | 8.82 | 51.80 | 30.86 | 1.89 |
| BG | 10.83 | 5.74 | 2.05 | 2.78 | 22.09 | 8.28 | 1.60 |

DAM = Dehydrated alfalfa meal; DBP = Dehydrated beet pulp.
BG = Barley grain; CP = Crude protein; CF = Crude fibre.
EE = Ether extract; NDF = Neutral detergent fibre.
ADF = Acid detergent fibre; ADL = Acid detergent lignin.

O'Donovan, 1995) recently reviewed by Omed et al. (2000) showed that the faeces can represent a valid alternative source of inoculum to estimate *in vitro* digestibility as proposed by Tilley and Terry (1963). In particular, O'Donovan (1995) in his study on inocula from faeces of six different species, also noted that the dry matter digestibility of different hays and straw measured *in vitro* with rabbit faeces diluted in phosphate buffer was significantly related to *in vitro* digestibility of the same roughage obtained using faecal inoculum from sheep ($r^2$ = 0.96 and 0.88, respectively for hays and straw). The latter was, in turn, significantly related to *in vitro* digestibility measured with rumen liquor of sheep ($r^2$ = 0.91) as well as with the apparent digestibility measured *in vivo* ($r^2$ = 0.98).

On comparing bovine rumen liquor and faeces as inoculum for the study of fermentation kinetics of some roughages with IVGPT, Mauricio et al. (2001) recorded less gas production with faecal inoculum, which was attributed to a different microbial population present in the faeces. Cutrignelli et al. (2005), in a similar trial in which used inocula (rumen liquor and faeces) from buffalo and roughage and concentrates as substrates, obtained higher gas production from faeces.

The aim of our research was to compare the fermentative activity of caecal content and faeces of rabbits used as a source of inoculum in a IVGPT conducted on three feedstuffs, commonly used as ingredients in rabbit diets (dehydrated alfalfa meal, dehydrated beet pulp and barley). Feed ingredients were chosen to test the fermentation activity of rabbit hindgut microflora in relation to different source of carbohydrates (structural and lignified, alfalfa; structural but easy fermentable, beet pulp; non structural, barley) without pre-digestion by protease.

## MATERIAL AND METHODS

### Substrate preparation

Three feedstuffs (dehydrated alfalfa meal, DAM, *Medicago sativa*; dehydrated beet pulp, DBP, *Beta vulgaris*; barley, BG, *Hordeum vulgare*) were used as substrates. The feedstuffs were ground to pass a 1 mm screen (Brabender Wiley mill, Brabender OHG Duisburg, Germany) and their chemical composition (Table 1) was determined (AOAC, 2000).

Cumulative gas production was measured according to the IVGPT method proposed by Theodorou et al. (1994). For each substrate, about 1 g of sample (in quadruplicate per inoculum) was weighed in a 120 ml serum flask and 75 ml of anaerobic buffered modified medium D (Theodorou, 1993) and 4 ml of reducing solution were added. Three bottles per inoculum were prepared without substrate and were used as "blank" to correct data relative gas production, organic matter degradability and volatile fatty acid production. The bottles were sealed with butyl rubber stoppers and aluminium crimp seals and incubated at 39°C until inoculation.

### Inocula preparation

The sampling of inocula (caecal content and faeces) was made in the morning in a specialised slaughter house on 25 hybrid Hyla rabbits raised on a commercial farm near Avellino (Italy), weighing an average 2.43±0.53 kg. From the 56$^{th}$ to 77$^{th}$ day of age (the latter being the slaughter date) the rabbits were fed *ad libitum* a commercial finisher diet (on DM basis: CP 15.7%, EE 3.1%, Ash 9.1%, NDF 35.0%, ADL 4.5%). From the night before slaughter, the animals fasted, but the water was available, as usually made for all the species before slaughter.

Once the whole gastro-intestinal tract had been isolated, the caecal content and faeces were collected and put into a pre-warmed thermos, filled to the brim in order to keep air content to a minimum. After sampling, the material was transported as soon as possible (about 2 h) to our department laboratories.

In the laboratory, 100 ml of caecal content were diluted with 100 ml of anaerobic medium, stirred for 5 minutes and strained through six layers of muslin under $CO_2$. The retained solids were then mixed with 100 ml of medium and homogenised in a blender for 20 s under $CO_2$. The homogenate was then re-strained through six layers of muslin; the resulting liquid was combined with the other strained fluid and held at 39°C under $CO_2$ until use (final dilution 2:1 medium:caecal content).

The fresh faeces (100 g) were added with 400 ml of anaerobic medium, stirred and strained through six layers of muslin. The remaining solids were then re-suspended in 400 ml of medium and homogenised by blending for 20 s. The homogenate was strained through six layers of muslin, mixed with the first strained solution and held at 39°C under $CO_2$ until use (final dilution 8:1 medium:faeces). The large faeces dilution was necessary owing to obtain a better separation of bacteria (Omed et al., 2000) and to simplify the introduction of the inoculum in the flasks.

The time taken for preparation of caecal and faeces inocula was around 30 min. A syringe fitted with an 18 gauge (1.2 mm) needle was used to inject 10 ml of caecal or faecal fluid into each flask. Before inoculation, the

**Table 2.** Some characteristics of rabbit caecal content (CC) and faeces (F)

| | DM (%) | Ash (%) | pH | VFA (mM) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Acetate | Prop | Butyr | Isobutyr | Valerianic | Isovalerianic | Total |
| CC | 24.59 | 3.43 | 6.30 | 40.56 | 7.13 | 9.84 | 1.27 | 0.05 | 0.16 | 59.01 |
| F | 34.36 | 4.59 | 7.90 | 1.46 | 0.18 | 0.27 | 0.03 | ND | ND | 1.94 |

DM = Dry matter, VFA = Volatile fatty acid; ND = Not detected.

displaced gas was allowed to escape and after inoculation the bottles were placed in an incubator at 39°C for 96 h.

Samples of caecal content and faeces were analysed for dry matter and ash (AOAC, 2000) pH and volatile fatty acid (VFA) content.

**Gas measurements and analysis at end of incubation**

Gas production was recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 21, 24, 27, 31, 38, 45, 48, 51, 57, 72 and 96 h post-inoculation. Initial readings were taken at two-hour intervals due to the rapid rate of gas production. The gas measurements were made using a pressure transducer connected to a three-way stopcock. The first outlet was connected to the pressure transducer, the second to a disposable plastic syringe and the third to a 23 gauge (0.6 mm) needle. Pressure readings (Pa) were taken by inserting the needle, connected to the three-way stopcock, through the stopper by withdrawing the accumulated gas in a syringe until the transducer display unit showed zero (equal to ambient pressure) and the volume of gas produced was measured. The gas was discarded and the bottles, after stirring, returned to the incubator. At the end of incubation (96 h), the bottles were placed at 4°C to terminate fermentation. The pH of each bottle was recorded (Alessandrini Instrument glass electrode, Jenway, Dunmow, UK; model 3030) and a sample of about 10 ml of liquid was collected and frozen prior to Volatile Fatty Acid (VFA) analysis. Substrate degradability was estimated by filtering the residues using preweighed sintered glass crucibles (Scott Duran, porosity 2) under vacuum. Residue dry matter was determined by drying to a constant weight at 103°C, and OM by difference following ashing (5 h at 550°C). Gas volumes obtained were related to the quantity of incubated (OMCV) and degraded (YOM) organic matter.

VFA analysis was made, after centrifugation and dilution of the samples with oxalic acid (1:1 v/v) by the gas-chromatography method (Thermo Electron, Italy mod. 8,000 top, FUSED SILICA Gaschromatograph with OMEGAWAX 250 fused silica capillary column SUPELCO, Italy 30 m×0.25 mm×0.25 mm film thickness; analysis temperature 175°C; flame ion detector 240°C; carrier Helium 1.5 ml/min).

**Curve fitting and statistical analysis**

The data from cumulative gas production were fitted to the equation of Groot et al. (1996):

$$G(t) = A/[1+(B/t)^c]$$

where G (ml/g OM) is the amount of gas produced per gram of organic matter incubated; A (ml/g OM) is the potential gas production; B (h) is the time after incubation at which half of A has been reached; C is a constant determining the curve sharpness. The maximum degradation rate (Rmax, ml/h) and the time at which it occurs (Tmax, h) were calculated according to the following equations (Bauer et al., 2001):

$$R = \frac{A \times B^C \times Tmax^{-C-1}}{(1+B^C \times Tmax^{-C})^2}$$

$$Tmax = B \frac{C-1}{(C+1)^{\frac{1}{C}}}$$

All the fermentative characteristics were analysed by "two-way" ANOVA (SAS, 2000) using the model:

$$Y_{ijk} = \mu + S_i + I_j + SI_{ij} + \varepsilon_{ijk}$$

where Y is the single observation; μ is the general mean; S is the substrate effect (i = alfalfa hay, beet pulp, barley); I is the inocula effect (j = caecum or faeces); SI is the interaction between the effects; and ε is the error.

For each fermentation parameter, the values obtained with caecal inoculum were estimated from the correspondent values from fecal inoculum (independent factor) by PROC REG (SAS, 2000).

## RESULTS

**Caecal content and faeces characteristics**

Table 2 reports some chemical characteristics as well as the pH and volatile fatty acid proportions of rabbit caecal content (CC) and faeces (F). The highest dry matter content of F (34.36 vs. 24.59%) more justify the use of a large medium proportion to obtain a satisfactory separation of the micro-organisms from digesta (Omed et al., 2000). The volatile fatty acid (VFA) contents obtained from F are much lower than those recorded in CC even if, expressed as percentages of total VFA, they do not appear to differ much between the two inocula (Acetate 68.7 vs. 75.0%; Propionate 12.1 vs. 9.28%; Butyrate 16.7 vs. 13.9%,

**Table 3.** *In vitro* fermentation characteristics with the two rabbit inocula for the three substrates

| | OMd (%) | OMCV (ml/g) | YOM (ml/g) | A (ml/g) | B (h) | Rmax (ml/h) | Tmax (h) |
|---|---|---|---|---|---|---|---|
| Inoculum (DF = 1) | | | | | | | |
| CI | 66.75 (18.15) | 256.7 (77.26) | 400.0 (51.62) | 266.6 (65.38) | 31.68 (9.18) | 6.76 (2.63) | $15.53^{a}$ (3.93) |
| FI | 67.58 (17.10) | 267.1 (82.33) | 383.6 (67.21) | 276.8 (87.84) | 37.35 (17.04) | 7.98 (3.16) | $11.96^{b}$ (4.09) |
| Substrate (DF = 2) | | | | | | | |
| DAM | $43.81^{C}$ (2.55) | $159.2^{C}$ (21.05) | $365.0^{b}$ (58.82) | $172.8^{B}$ (22.42) | $45.69^{A}$ (9.84) | $2.50^{C}$ (0.62) | $11.00^{B}$ (3.07) |
| DBP | $75.27^{B}$ (2.01) | $290.8^{B}$ (24.78) | $386.1^{ab}$ (28.89) | $310.7^{A}$ (27.04) | $37.68^{A}$ (5.38) | $5.48^{B}$ (0.80) | $19.05^{A}$ (3.72) |
| BG | $82.41^{A}$ (1.65) | $349.2^{A}$ (56.11) | $424.3^{a}$ (71.81) | $331.6^{A}$ (25.90) | $20.19^{B}$ (4.54) | $14.12^{A}$ (3.43) | $11.18^{B}$ (4.57) |
| Significance | | | | | | | |
| I | NS | NS | NS | NS | NS | NS | * |
| S | ** | ** | * | ** | ** | ** | ** |
| I×S | NS | NS | NS | NS | NS | NS | NS |

CI = Caecal inoculum; FI = Faecal inoculum; DAM = Dehydrated alfalfa meal; DBP = Dehydrated beet pulp; BG = Barley.
OMd = Organic matter degradability; OMCV = Cumulative volume of gas by incubated organic matter.
YOM = Cumulative gas production by degraded organic matter; A = Potential gas production; B = Time at which A/2 is produced.
Rmax = Maximum fermentation rate; tRmax = Time at which Rmax is reached.
I = Inoculum effect; S = Substrate effect; I×S = Interaction between the effects.
$^{A, B, C,}$ ** $p<0.01$; $^{a, b,}$ * $p<0.05$; NS = not significant, (…) = standard deviation.

**Table 4**. End products of fermentations

| | pH | Acetate | Propionate | Butyrate | Isobutyrate | Valerianic | Isovalerianic | tVFA |
|---|---|---|---|---|---|---|---|---|
| | | | | | mM/g SOi | | | |
| Inoculum (DF = 1) | | | | | | | | |
| CI | 6.51 (0.20) | $38.90^{A}$ (4.46) | $7.12^{B}$ (1.38) | $11.26^{B}$ (0.97) | $0.74^{A}$ (0.24) | 0.62 (0.41) | 0.68 (0.26) | 59.31 (6.09) |
| FI | 6.52 (0.17) | $33.38^{B}$ (6.28) | $10.33^{A}$ (6.10) | $13.96^{A}$ (5.77) | $0.42^{B}$ (0.30) | 0.73 (0.45) | 0.52 (0.49) | 59.34 (18.80) |
| Substrate (DF = 2) | | | | | | | | |
| DAM | $6.73^{A}$ (0.09) | $28.51^{B}$ (3.77) | $4.19^{B}$ (1.49) | $8.37^{B}$ (1.70) | $0.31^{B}$ (0.28) | $0.70^{B}$ (0.34) | $0.33^{b}$ (0.17) | $42.41^{B}$ (7.52) |
| DBP | $6.34^{C}$ (0.05) | $39.08^{A}$ (2.68) | $11.48^{A}$ (4.48) | $14.83^{A}$ (3.67) | $0.54^{AB}$ (0.23) | $0.50^{B}$ (0.19) | $0.62^{ab}$ (0.49) | $67.05^{A}$ (7.15) |
| BG | $6.46^{B}$ (0.07) | $40.40^{A}$ (2.31) | $11.87^{A}$ (3.62) | $16.09^{A}$ (4.10) | $0.88^{A}$ (0.12) | $1.11^{A}$ (0.74) | $1.01^{a}$ (0.16) | $71.36^{A}$ (6.30) |
| Significance | | | | | | | | |
| I | NS | ** | ** | ** | ** | NS | NS | NS |
| S | ** | ** | ** | ** | ** | ** | * | ** |
| I×S | NS | NS | ** | ** | NS | ** | NS | NS |

CI = Caecal inoculum; FI = Faecal inoculum; DAM = Dehydrated alfalfa meal; DBP = Dehydrated beet pulp; BG = Barley.
OMd = Organic matter degradability; OMCV = Cumulative volume of gas by incubated organic matter.
YOM = Cumulative gas production by degraded organic matter; A = Potential gas production; B = Time at which A/2 is produced.
Rmax = Maximum fermentation rate; tRmax = Time at which Rmax is reached.
I = Inoculum effect; S = Substrate effect; I×S = interaction between the effects.
$^{A, B, C,}$ ** $p<0.01$; $^{a, b,}$ * $p<0.05$; NS = Not significant, (…) = Standard deviation.

respectively for CC and F). In each case, the average characteristics of rabbit caecal content are in agreement with those reported by other authors (Gidenne, 1995; Piattoni et al., 1998). As regards faeces, our results were partly expected since in the caecum and in the colon part of the water and VFA are absorbed.

**Fermentation characteristics**

Table 3 shows the fermentation characteristics by inoculum and substrate. As noted, between the inocula statistically significant ($p<0.05$) differences occur only for the time at which the maximum rate was reached (Tmax), higher for caecal inoculum (15.53 vs. 11.96 h). Except YOM, all the other parameters, albeit without significant differences, were already higher with faecal inoculum (FI).

The true and potential gas production, OMd and Rmax, according to chemical composition, allows the substrates to be classified as follows: BG>DBP>DAM showing in each case statistically significant ($p<0.01$) differences. Our results agree with those reported by Gazaneo et al. (2003) who tested the same substrates in a trial with the IVGPT using caecal content of rabbits around weaning as inoculum.

Tmax of DBP (11.0 h) was lower ($p<0.01$) than that of DAM (19.05) and similar to BG (11.18). This result is also in agreement with Gazaneo et al. (2003) and could be due to the fact that the micro-organisms are able to degrade only the easily fermentable carbohydrates in the alfalfa, as shown by the OMd which slightly exceeds 40%.

In each case, the inocula x substrate interaction was not statistically significant. To better view the different trends of inocula by substrates, it is worth representing in graphs the cumulative gas production (Figure 1) and the

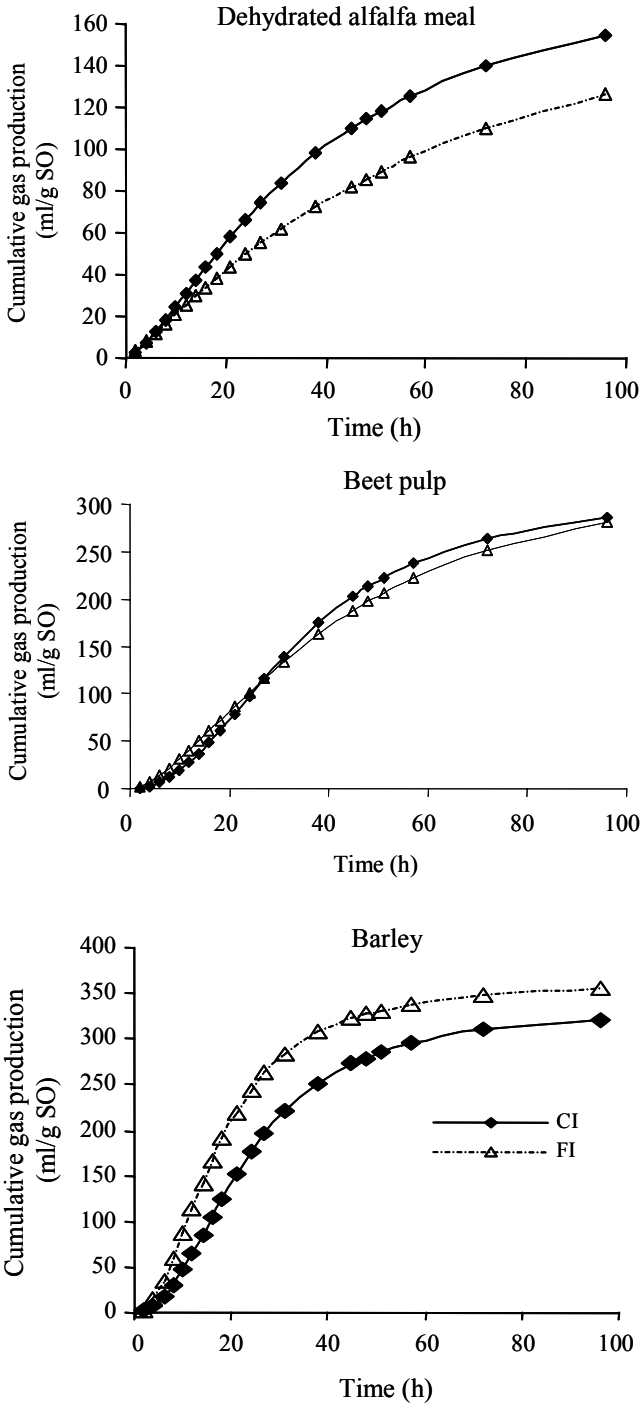


**Figure 1.** Gas production of the three feedstuffs by inoculum over time. CI = Caecal inoculum; FI = Faecal inoculum.

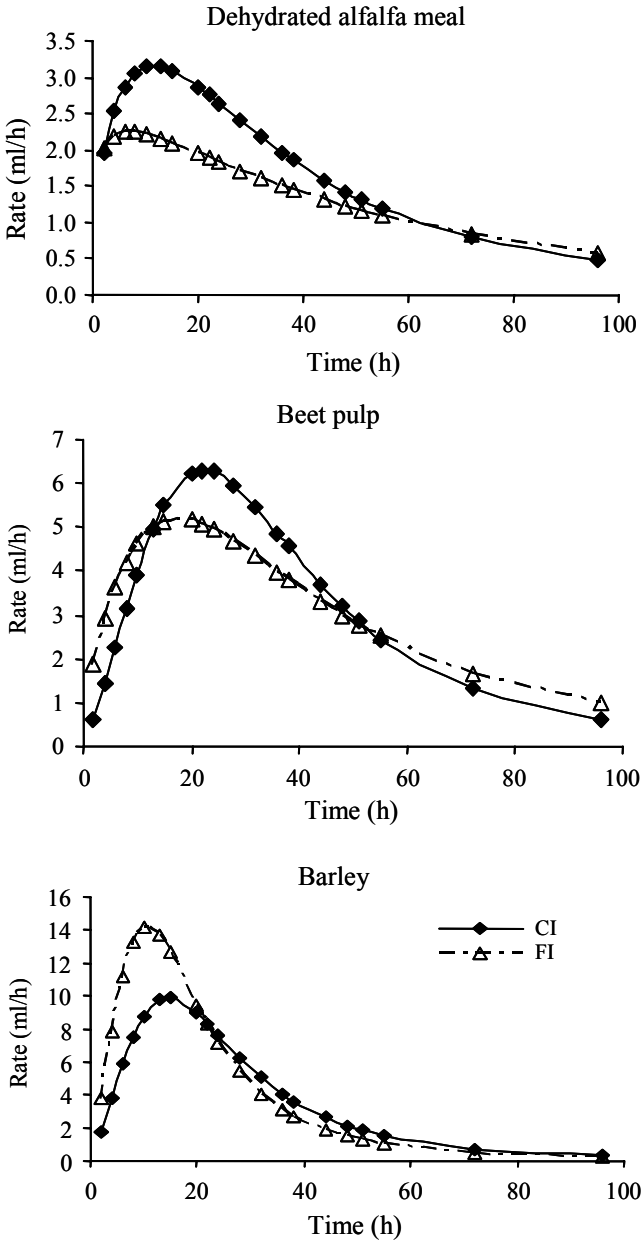


**Figure 2.** Fermentation rate of the three feedstuffs by inoculum over time. CI = Caecal inoculum; FI = Faecal inoculum.

fermentation rate (Figure 2). Importantly, for each reading, a mean value obtained from four bottles was used to generate gas production profiles and to estimate gas production parameters.

**End products of the fermentation**

Table 4 reports pH and VFA contents at the end of fermentation. In DBP, the high content of easily fermentable structural carbohydrates results in pH values significantly ($p<0.01$) lower than DAM and BG.

The two inocula were very different in terms of VFA production. The CI produced higher levels of acetate (38.9 vs. 33.4 mM/g OM incubated, $p<0.01$) and isobutyrate (0.72 vs. 0.42, $p<0.01$) but lower concentrations of propionate (7.1 vs. 10.3, $p<0.01$) and butyrate (11.3 vs. 14.0, $p<0.01$). However, total VFA content was not significantly different between the inocula. The substrates also showed significant differences in VFA production. Thus, DAM had the lowest total VFA production and BG the highest. Moreover, for propionate, butyrate and valerianic acid the interaction between the effects was also statistically

**Table 5.** Average values (mM/g SOi) of propionate, butyrate and valerianic acid by feed and inoculum

| | | Propionate | Butyrate | Valerianic |
|---|---|---|---|---|
| DAM | CI | 5.10 | 9.83 | 1.03 |
| | FI | 3.28 | 6.89 | 0.37 |
| DBP | CI | 8.00 | 11.84 | 0.49 |
| | FI | 14.96 | 17.83 | 0.51 |
| BG | CI | 7.82 | 12.63 | 0.74 |
| | FI | 15.93 | 19.54 | 1.48 |

DAM = Dehydrated alfalfa meal; DBP = Dehydrated beet pulp.
BG = Barley; CI = Caecal inoculum; FI = Faecal inoculum.

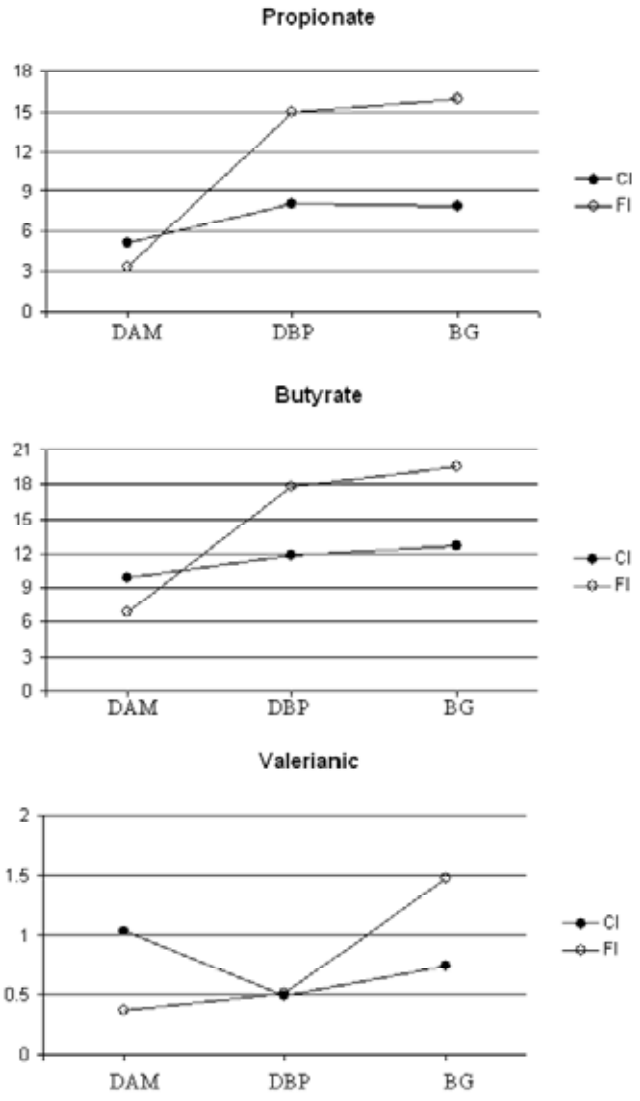


**Figure 3.** Multiple comparison for interaction between inocula and substrates. CI = Caecal inoculum; FI = Faecal inoculum; DAM = Dehydrated alfalfa meal; DBP = Dehydrated beet pulp; BG = Barley grain.

significant ($p<0.01$). To clarify this result Table 5 reports the average data for each substrate and inoculum of the three VFA; in Figure 3 the multiple comparison for interaction between inocula and substrates are showed.

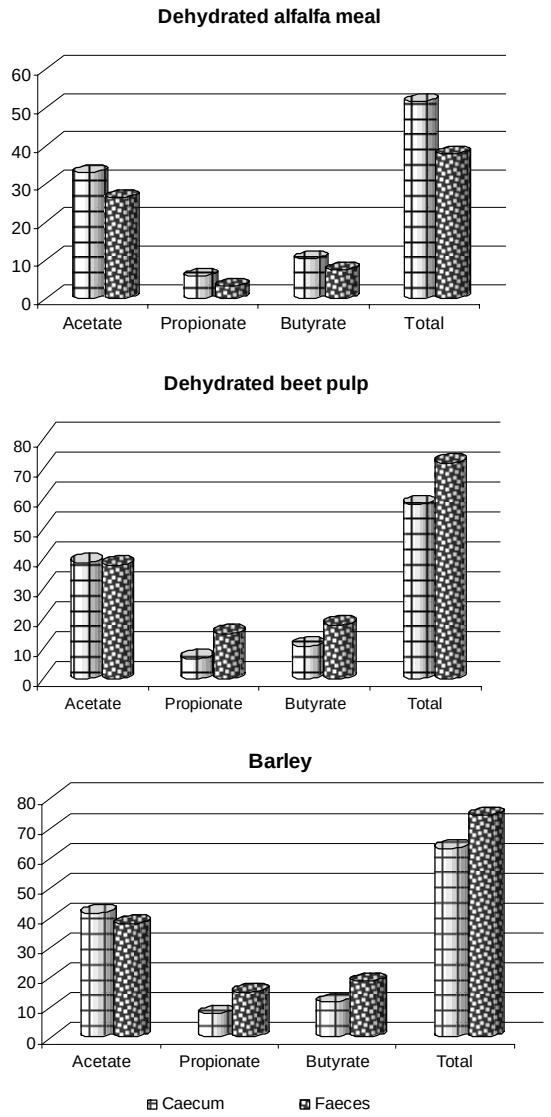


**Figure 4.** Volatile fatty acid (mM/g SOi) the three substrate with the two inocula.

To better understand the VFA trend from substrates and inocula, we used Figure 4. The CI, in all the tested feeds, induced a higher production of acetate and, in the case of alfalfa, also of most important VFAs so that, for this substrate, total VFA production was higher than FI. When it compared the two inocula incubated with DBP and BG, total VFA production was higher with FI. A major contribution to this result comes from propionate and butyrate.

**Estimation of caecal fermentation characteristics by faeces**

Table 6 reports the regression equations for the estimation of the caecum fermentation characteristics by faeces. For the sake of brevity, we report only the parameters for which r was statistically significant.

**Table 6.** Equations for estimation of caecal parameters from faeces

| CI | FI | RSD | P values |
|---|---|---|---|
| OMd | -4.31(3.18)+1.05(0.045) OMd | 2.59 | <0.001 |
| OMCV | 116.04 (45.55)+0.56 (0.16) OMCV | 54.34 | 0.0057 |
| pH | -0.045 (1.17)+1.005 (0.18) pH | 0.10 | 0.0002 |
| tVFA | 41.80 (4.59)+0.28 (0.07) tVFA | 3.28 | 0.0097 |
| Acetate | 18.46(5.54)+ 0.60 (0.16) Acetate | 2.50 | 0.0132 |
| Prop | 5.39(1.00)+0.15 (0.08) Prop | 1.14 | 0.0497 |
| Buty | 9.37 (0.80)+0.12 (0.05) Buty | 0.71 | 0.0098 |

CI = Caecal inoculum; FI = Faecal inoculum; OMd = Organic matter degradability; OMCV = Cumulative volume of gas per gram of organic matter; tVFA = Total volatile fatty acid; Acet = Acetate; Prop = Propionate; Buty = Butyrate; (…) = Standard error.

## DISCUSSION

The results showed clearly that there are important differences in the fermentation characteristics of different substrates with CI or FI, but many of these are significantly related.

Unfortunately, the lack of data on the microbial population of rabbit faeces in the literature suggests that some hypotheses be formulated on the basis of studies on faeces of other poly- or mono-gastric species. Allison (1984) found no differences in terms of micro-organism number among the population of rumen liquor and faeces despite some previous findings (Hobson, 1971; Kern et al., 1974; Sharpe et al., 1975) that the microbial population of faeces was lower than those of the rumen; the same authors showed that micro-organisms were present in the hind gut and in faeces in similar number. Thus, the microbial populations of caecal content and faeces of rabbit may be held to be very similar. As confirmation of this hypothesis, Houdijk (1998) in a study on the effects of non-digestible oligosaccharides on microbial composition of ileum and caecal contents and faeces of swine around weaning, found no major differences in bacterial cell count on caecal content and faeces. In our case, the differences in fermentation characteristics of the different feeds by inoculum seem to indicate a different composition or, probably, a different activity of bacterial populations, lower for cellulolytic micro-organisms. It can be clarify looking at the different trend of inocula according to tested feeds (Figures 1 and 2).

For the three substrates it may be observed that the gas production curves (Figure 1) obtained with faeces (FI) and caecal inoculum (CI) show a similar trend. For alfalfa the gas production was at each time higher with CI than FI, according to Mauricio et al. (2001) who compared bovine faeces and rumen liquor. Moreover, the DAM and DBP gas production curves did not decrease at the end of fermentation: more than 96 h of incubation would probably need to obtain the complete fermentation of this substrates. For beet pulp the gas profiles with the two inocula were very similar. According to the barley graph, the FI showed a higher gas production over time than CI.

By contrast, the fermentation rate profiles (Figure 2) had a different trend for the three substrates:

• DAM: the FI fermented the substrate slower than CI at each time; the curve shape is rather flat and failed to show the typical parabola;

• DBP: the FI was faster than the CI in the first 10 h when it was surpassed by the CI that reached the highest Rmax;

• BG: until 20 h the FI showed a higher fermentation rate than the CI. The trend changes when the process is beginning to finish.

As consequence of this considerations, we might hypothesize, resulting from less cellulolytic bacterial activity, more intense fermentation of non-structural carbohydrates (very high in barley) that induce a rapid pH decrease (then partly buffered by medium) and further hinder the activity of cellulolytic bacteria since, as reported by Ørskov (1982), a pH of less than about 6.2 will seriously inhibit cellulolytic bacterial growth.

Given also the major differences between caecal content and faeces in terms of volatile fatty acid production (Table 2) we can conclude, in agreement with Mauricio et al. (2001), that the micro-organisms in the faeces are likely to be in a state of "suspended animation", similar to the "somnicell state" described by Roszak and Colwell (1987). The state of suspended animation is brought about by a number of environmental factors: most important are the low substrate availability and the higher oxygen concentration at which cellulolytic bacteria are, probably, more sensitive. Moreover, Corbett (1981), in a study on bovine rumen liquor and faeces as inocula for an *in vitro* digestibility trial on roughages, found that faecal fluid contains insufficient trace elements and micro-nutrients, reinforcing the effect of forage deficiencies on digestibility.

Evermore regarding VFA, our results are in agreement with Garcia et al. (2002) that reported in growing rabbits a progressive reduction in caecal pH when digestible NDF percentage in feeds increases.

Looking at the Figure 3, it is possible to note that the significant interaction between inocula and substrates for propionate, butyrate and valerianic acid is due to the fact that for each VFA DAM showed lower and DBP and BG higher values, with FI than CI, respectively. It confirms a different behaviour of the two inocula in relation to the

source of carbohydrates tested in the *in vitro* gas production trial.

The results obtained from faecal inoculum show a higher variability than those from caecal content. Again in agreement with Mauricio et al. (2001), this could confirm the lack of microbial activity of the faecal.

As noted, the regression between kinetic parameters, obtained after fitting of experimental data with mathematical models, was not statistically significant (according to Mauricio et al., 2001 and Altaf et al., 1998 that compared rumen liquor and faeces) while the parameters reported in table V were positively related and, except for propionate, statistical significance reached 1%. Regarding total VFAs, propionate and butyrate the regression coefficient was so low as to indicate little influence on the estimation of the corresponding parameter by faeces.

## CONCLUSIONS

The IVGPT is confirmed as a very good technique, adaptable to poly- and monogastric animals, which can also be used to study fermentation characteristics of different gastro-intestinal tracts.

As regards faecal inoculum, the similar trend of gas production and fermentation rate and the existence of significant regressions between some fermentation parameters suggest that, also in rabbit, despite the different microbial activity, faeces could be used as an alternative source of inoculum to study the fermentation characteristics of feed or the fermentation activity of the caecal microbial population.

Our study represents a preliminary approach to this topic and undoubtedly further studies are required along specific lines. First, the interaction between inoculum and feed effects emerging for some VFA suggests that it would be opportune to make IVGPT tests by feedstuff categories (roughage and concentrate) in order to obtain more precise estimating equations. A further problem to solve is the method of faeces collection. In rabbits, while it is not possible to collect faeces directly from the rectum (very easy in ruminants), the faeces could be collected on evacuation in order to prevent contamination by environmental bacteria and excessive exposure to air that can modify the activity of the normal faecal microbial population.

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