

# Use of the *in vitro* gas production technique to study feed digestibility in domesticated ostriches (*Struthio camelus* var. *domesticus*)

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In order to enhance knowledge of the nutritive value of ostrich feeds, the *in vitro* gas production technique was used. Five feeds (corn silage, CS; alfalfa hay, AH; barley, BG; soybean meal, SM; beet pulp, BP) were weighed (~ 1g) in quadruplicate in 120 ml serum flasks and incubated at 39 °C with 75 ml of anaerobic medium and 4 ml of reducing solution. A pool of caecal contents obtained at slaughter from 4 male ostriches was diluted 1:2 with the medium and used as inoculum (10 ml/flask). Gas production was recorded at regular intervals up to 120 h from inoculation. At the end of incubation, pH and organic matter digestibility (OMd) were measured. The gas produced at each time was fitted to a mathematical model to obtain the kinetics fermentation parameters (e.g. maximum fermentation rate, Rmax and time at which it occurs, Tmax). On the basis of OMd, the substrates were classified as: AH (55.5 %) < CS (63.82 %) < BG (79.98 %) < BP (80.78 %) < SM (88.51 %). BG and BP showed the same pH (6.36) and similar gas production (OMCV, 321.9 ml/g vs 317.0, respectively for BG and BP). However, BG showed higher Rmax (9.83ml gas/h vs 7.23, respectively for BG and BP, P < 0.0001) and lower Tmax (17.51 h vs 22.36, respectively for BG and BP, P<0.0001). SM, also showing the highest OMd, showed a low gas production (OMCV, 258.2 ml/g), confirming that not all fermented organic matter produces gas. The OMd of AH and BG was in agreement with the findings of another study in which the dry matter digestibility in ostrich of alfalfa (50.1 %) and barley (82.6 %) was determined by *in vivo* trials. Our results suggest that the *in vitro* gas production technique can be used successfully to estimate the organic matter digestibility of ostrich feed and study the fermentation characteristics of ostrich caecal content.

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**Keywords:** ostrich; *Struthio camelus*, *in vitro* gas production; feed digestibility

## Introduction

To obtain a useful feed value, it is necessary to determine digestibility, in particular for ostriches where the nutritive value of feeds used for diet formulation is very often erroneously determined using poultry: the ostrich caecum provides a suitable environment for the fermentation of dietary fibre, such that the ostrich is considered to be a monogastric herbivore, like the rabbit.

Some authors (Cilliers et al., 1997) determined the feed digestibility of ostriches *in vivo*, using an ingesta-excreta balance method. Laboratory analysis of feeds may include measuring *in vitro* digestion. *In vitro* methods are much easier to perform and cheaper than conventional digestibility trials which are also time-consuming. Recently, Nheta et al. (2005), studying the *in vitro* organic matter digestibility of ostrich diets using the normal and reverse Tilley and Terry (1993) method, concluded that the method, commonly used for ruminants, needs to be modified to suit ostriches.

Since the 1990s there has been increasing interest in the *in vitro* technique which measures gas production both to study the kinetics of rumen fermentations (Blümmel et al., 1997; Getachew et al., 1998; Calabrò et al., 2002) and estimate the *in vivo* digestibility of ruminant feeds (Menke and

Steingass, 1988; Blümmel et al., 1993). Given the valid results obtained and the relatively straightforward low-cost trials, the in vitro gas production technique (IVGPT) was recently also used in other species such as rabbits (Calabrò et al., 1999; Gazaneo et al., 2003; Stanco et al., 2003) to study the caecal environment and feed digestibility.

The IVGPT is based on the fact that the anaerobic digestion of carbohydrates by rumen or caecal micro-organisms produces gas ( $\text{CO}_2$ ,  $\text{CH}_4$  and traces of  $\text{H}_2$ ) 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).

The aim of our research was to study the fermentative activity of the caecal content of ostriches used as a source of inoculum in an IVGPT conducted on five feedstuffs, commonly used as ingredients in ostrich diets (corn silage, alfalfa hay, barley, soybean meal, beet pulp). The simple feeds were chosen in order to study the fermentation activity of ostrich hindgut microbial populations on various sources of carbohydrates and protein.

## Material and method

Five feeds (corn silage, CS, *Zea mais*; alfalfa hay, AH, *Medicago sativa*; barley, BG, *Hordeum vulgare*; soybean meal, SM, *Glycine max*; beet pulp, BP, *Beta vulgaris*) 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 (Tab. 1) was determined (AOAC, 2000).

Cumulative gas production was measured according to the IVGPT method proposed by Theodorou et al. (1994). For each substrate,  $1.0057 \pm 0.0036$  g of sample (in quadruplicate) 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 flasks were prepared without substrate and were used as “blanks”. The flasks were sealed and incubated at 39°C until inoculation.

The caecal content was sampled in the morning in a specialised slaughter house on 4 male ostriches raised on a commercial farm in Naples (Italy), weighing an average  $95.6 \pm 3.12$  kg. From the 4<sup>th</sup> to 10<sup>th</sup> month of age (the latter being the slaughter date) the ostriches were fed ad libitum a diet consisting of dehydrated alfalfa (40 % DM), commercial concentrate (35 %) and a cereal mix (25 %). From the night before slaughter, the animals fasted, although water was available.

Once the whole gastro-intestinal tract had been isolated, the caecal content was 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 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 gauze under  $\text{CO}_2$ . The retained solids were then mixed with 100 ml of medium and homogenised in a blender for 60 s under  $\text{CO}_2$ . 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  $\text{CO}_2$  until use (final dilution 2:1 medium:caecal content).

The time taken for preparing inoculum was around 30 min. A syringe fitted with an 18 gauge (1.2 mm) needle was used to inject 10 ml of caecal fluid into each flask. Before inoculation, the displaced gas was allowed to escape and after inoculation the flasks were placed in an incubator at 39°C for 120 h.

Gas production was recorded at 2, 4, 6, 9, 12, 14, 16, 19, 21, 24, 27, 33, 36, 40, 44, 48, 52, 60, 68, 72, 96 and 120 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 (PSI) 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 (120 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). 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 digestibility (OMd) 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.

The data from cumulative gas production were fitted to the equation of Groot et al. (1996):  $G(t) = \frac{A \times B^C \times T_{\max}^{-C-1}}{(1 + B^C \times T_{\max}^{-C})^2}$ , 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 ( $R_{\max}$ , ml/h) and the time at which it occurs ( $T_{\max}$ , h) were calculated according to the following equations (Bauer et al., 2001):

$$R_{\max} = \frac{A \times B^C \times T_{\max}^{-C-1}}{(1 + B^C \times T_{\max}^{-C})^2} \quad T_{\max} = B \times \frac{C-1}{(C+1)^{\frac{1}{C}}}$$

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

$$Y_{ijk} = \mu + S_i + \varepsilon_{ijk}$$

where:

$Y$  is the single observation;

$\mu$  the general mean;

$S$  the substrate effect ( $i$  = corn silage, alfalfa hay, barley, soybean meal or beet pulp);

$\varepsilon$  the error.

## Result and discussion

Table 1 shows the chemical composition of the five feeds used for the in vitro gas production trial. On the basis of the structural carbohydrate (NDF) amount, the five feeds may be classified as high fibre (AH and BP), medium fibre (CS) and low fibre (BG and SM).

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

	DM, %	CP	CF	EE	Ash	NDF	ADF	ADL
AH	90.00	13.10	29.04	1.31	7.53	47.55	44.48	9.82
BP	88.13	9.32	20.18	0.95	5.15	51.80	30.86	1.89
CS	33.93	8.26	21.51	2.15	5.95	37.26	29.24	4.12
BG	87.20	9.62	6.77	1.49	3.29	22.09	8.28	1.60
SM	87.49	43.19	6.61	2.13	6.53	14.75	9.29	0.81

AH = alfalfa hay; BP = beet pulp; CS = corn silage; BG = barley grain; SM = soybean meal; CP = crude protein, CF = crude fibre; EE = ether extract; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin

The results of the gas production trial (Table 2) are in agreement with the chemical composition of the feeds. Of great interest is the different behaviour of the two high-fibre feeds due to the different composition of the NDF. Indeed, AH, rich in lignified structural carbohydrates, showed significantly ( $P < 0.0001$ ) lower OMd (55.50 vs 80.78 %), OMCV (222.9 vs 317.0 ml/g OM) and  $R_{\max}$  (4.52 vs 7.23 ml gas/h) than BP whose structural carbohydrates were easy fermentable. However, the  $T_{\max}$  was significantly ( $P < 0.0001$ ) higher for BP than for AH (22.36 vs 4.53 h, respectively). This agrees with Gazaneo et al. (2003) and Bovera et al. (2006), and could be due to the fact that the micro-organisms are able to degrade essentially the easily fermentable carbohydrates of the alfalfa which, being in less quantity, quickly reached the maximum rate of fermentation.

CS, which may be considered halfway between forage and concentrate, showed an intermediate OMd (63.82 %), OMCV (248.1 ml/g),  $T_{\max}$  (16.08 h) and  $R_{\max}$  (5.50 ml/h).

Regarding the low-fibre feeds, soybean meal, despite having the highest OMd (88.51 %), showed a low gas production (OMCV, 258.2 ml/g; YOM, 291.7 ml/g and A, 229.2 ml/g) and the lowest  $T_{\max}$

(12.52 h). This is due to the high percentage of crude protein (43.19 %) which, though degraded by micro-organisms, does not contribute to gas production. Also in this case the few storage carbohydrates were rapidly ( $R_{max}$  4.95 ml gas/h) fermented and exhausted. The barley, rich in non-structural carbohydrates, showed the significantly ( $P < 0.0001$ ) highest  $R_{max}$  (9.83 ml/h) but the OMCV did not statistically differ from BP and, indeed, the potential gas production (A) was lower than BP (271.1 vs 281.5, respectively for BG and BP), although not statistically significant.

**Table 2** In vitro fermentation characteristics of the five substrates

	AH	BP	CS	BG	SM	MSE
OMd, %	55.50 <sup>D</sup>	80.78 <sup>B</sup>	63.82 <sup>C</sup>	79.98 <sup>B</sup>	88.51 <sup>A</sup>	1.82
OMCV, ml/g	222.9 <sup>B</sup>	317.0 <sup>A</sup>	248.1 <sup>B</sup>	321.9 <sup>A</sup>	258.2 <sup>B</sup>	180.1
YOM, ml/g	401.7 <sup>A</sup>	392.6 <sup>A</sup>	388.7 <sup>A</sup>	402.4 <sup>A</sup>	291.7 <sup>B</sup>	290.6
A, ml/g	192.8 <sup>C</sup>	281.5 <sup>A</sup>	224.5 <sup>B</sup>	271.1 <sup>A</sup>	229.2 <sup>B</sup>	55.61
B, h	26.70 <sup>B</sup>	30.02 <sup>A</sup>	27.02 <sup>AB</sup>	22.51 <sup>C</sup>	28.45 <sup>AB</sup>	1.41
Tmax, h	13.37 <sup>CD</sup>	22.36 <sup>A</sup>	16.08 <sup>BC</sup>	17.51 <sup>B</sup>	12.52 <sup>D</sup>	1.19
$R_{max}$ , ml/h	4.53 <sup>C</sup>	7.23 <sup>B</sup>	5.50 <sup>C</sup>	9.83 <sup>A</sup>	4.95 <sup>C</sup>	0.12
pH	6.68 <sup>A</sup>	6.36 <sup>C</sup>	6.41 <sup>B</sup>	6.36 <sup>C</sup>	6.68 <sup>A</sup>	0.0002

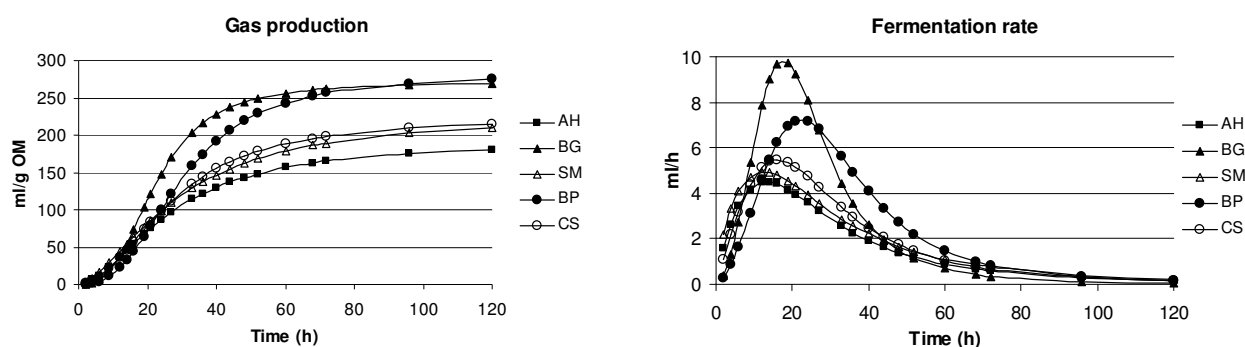
AH = alfalfa hay; BP = beet pulp; CS = corn silage; BG = barley grain; SM = soybean meal; OMd = organic matter digestibility; OMCV = organic matter cumulative volume; YOM = yield of organic matter; A = potential gas production; B = time at which  $\frac{1}{2}$  of A is produced; Tmax = time at which  $R_{max}$  is reached;  $R_{max}$  = maximum fermentation rate. A, B, C, D =  $P < 0.0001$ ; MSE = mean square error.

The pH values ensured in each case a favourable environment for cellulolytic bacteria activity (Doane et al., 1997). Interestingly, BP and BG showed the same pH at the end of fermentation (6.36).

Finally, excluding SM, gas production increased with organic matter digestibility. On the basis of the latter parameter the five feeds can be ranked as follows: AH (55.50 %) < CS (63.82 %) < BG (79.98 %) < BP (80.78 %) < SM (88.51 %).

The trend of gas production and fermentation rate is well described by Figure 1. As noted, barley produced a higher amount of gas than the other feeds. Also the fermentation rate was faster for BG even if the fermentations of the soluble carbohydrates of SM and AH start earlier. Finally, on the basis of both gas production and fermentation rate, the feed classification is: BG > BP > CS > SM > AH.

**Figure 1.** Gas production and fermentation rate of the five substrates



AH = alfalfa hay; BP = beet pulp; CS = corn silage; BG = barley grain; SM = soybean meal

Confirming the reliability of the IVGPT for studying the fermentation characteristics of feeds, our results also suggest that the technique can be reliably applied to study the fermentation activity of caecal contents and the organic matter digestibility of ostrich feeds.

More information on the fermentation characteristics of the ostrich caecum may well be obtained from analysing the volatile fatty acid composition, currently under way in our Department's laboratory.

As regards organic matter digestibility, our results agree with the finding of Cilliers et al. (1997) who, using a balance ingesta-excreta method, recorded dry matter digestibility of 50.1 % and 82.6 % for alfalfa and barley, respectively.

This suggests that the in vitro gas production technique can be successfully used to estimate the digestibility of ostrich feeds. This is of considerable importance since, compared with in vivo trials, the IVGPT is easier to perform, relatively inexpensive and also produces more reliable results.

In any case, further studies are required to expand the number of feeds analyzed in order to create a database containing data on gas production characteristics and organic matter digestibility of ostrich feeds and to ascertain the reliability of the IVGPT also for the study of the nutritive values of ostrich diets.

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