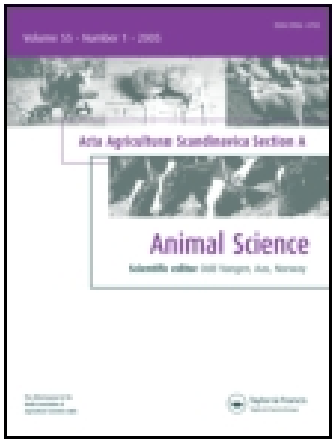

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ORIGINAL ARTICLE

Comparison of caecal and faeces fermentation characteristics of ostrich by *in vitro* gas production technique

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Abstract
To compare caecal and faecal fermentation characteristics, ostrich caecal content (CI) and faeces (FI) were used as *inocula* for an *in vitro* gas production trial in which four substrates (dehydrated alfalfa, alfalfa hay, maize and a commercial concentrate) were tested. The fermentation characteristics (degraded organic matter, OMd; potential gas production, *A*; acetate; branched chain proportion, BCP) were studied by *inoculum* and substrate. CI and FI showed significant differences for almost all the fermentation parameters, and CI had higher values than FI for OMd (76.83 vs. 72.79%, $p < 0.01$), *A* (250.3 vs. 229.3 ml/g, $p < 0.01$), acetate (57.91 vs. 53.20 mmol/l, $p < 0.01$) and BCP (0.031 vs. 0.027, $p < 0.05$). CI and FI showed differences in carbohydrates and protein fermentation, but the interaction between the tested effects was not significant. The regression equations to estimate caecal fermentation characteristics from faeces suggest the possibility to use faeces as *inoculum* alternative to faeces.

Keywords: *Caecal content, faeces,* in vitro *gas production technique, ostrich,* Struthio camelus.

Introduction

To ascertain the nutritive value of feedstuffs, it is necessary to measure their digestibility. This assertion particularly holds for ostriches. In fact, in this species the nutritive value of feeds is very often estimated from that of poultry. However, ostrich and poultry are very different in terms of feed digestion: in ostrich, the large intestine provides a suitable environment for the fermentation of nutrients and in particular structural carbohydrates (Musara et al., 2002), ostrich being a herbivore and not a granivorous bird.

While some authors (Cilliers et al., 1997) measured the feed digestibility of ostriches *in vivo*, using an *ingesta–excreta* balance method, *in vitro* methods to estimate feed digestibility are much easier to perform and cheaper than conventional digestibility trials which are also time-consuming. Of the *in vitro* methods, the *in vitro* gas production technique (IVGPT, Theodorou et al., 1994) is an invaluable method to study not only the fermentation characteristics of feeds (Getachew et al., 1998; Menke & Steingass, 1988) but also the physiology of some tracts of the digestive system, in particular the rumen in polygastric species (Calabrò et al., 2008) and the large intestine in monogastric animals (Calabrò et al., 2007). In single-stomached animals it is ascertained that the percentage of organic matter digestibility obtained by IVGPT without enzymatic pre-digestion is strongly related to the digestibility measured with the internal marker methods (Stanco et al., 2003).

The IVGPT is based on the fact that anaerobic digestion of carbohydrates by digestive micro-organisms produces gas (CO_2, CH_4 and traces of H_2) and volatile fatty acids (VFAs), mainly, acetate, propionate and 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 part of gastro-intestinal tract in which fermentations occur (*inoculum*).

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Recently, increasing attention to animal welfare has generated considerable ethical problems in carrying out such trials, in particular in species, such as the ostrich, where the collection of caecal content is tied to animal sacrifice. Hence, considerable research effort has been devoted to investigating the use of alternatives to rumen or large intestine fluid as a source of *inoculum*. Use of fresh faeces from cows and sheep, rather than rumen fluid, has been investigated for more than a decade as an alternative *inoculum* (Aiple et al., 1992; Omed et al., 2000), and its potential in the IVGPT has been reported by Harris et al. (1995), Mauricio et al. (2001) and Cone et al. (2002).

In single-stomached species, many researchers have used faecal *inocula*, partly to devise more appropriate *inocula* for studying hindgut fermentation. Thus, caecal fluid and faeces from broilers (Lan et al., 2007), pigs (Bindelle et al., 2007), horse (Murray et al., 2006) and rabbits (Bovera et al., 2006, 2008, 2009) have been used in avian, porcine, equine and rabbit nutrition, respectively. Also in ostriches, in which there is a separate excretion of urine and faeces (Duke et al., 1995), Bovera et al. (2007) evaluated the use of faeces as *inoculum* for gas production trials, and the organic matter digestibility obtained for alfalfa hay (55%) and barley (81%) were in agreement with those of Cilliers et al. (1997) who, using a balance *ingesta–excreta* method, found dry matter digestibility of 50 and 83% for alfalfa and barley, respectively.

The aims of this research were to compare the fermentation characteristics of the *inocula* from the caecal content and faeces of ostrich in a gas production trial and to evaluate to use faeces as IVGPT *inoculum* to measure ostrich feed digestibility.

Materials and methods

Substrate preparation

Four feeds (dehydrated alfalfa, DA, *Medicago sativa*, alfalfa hay, AH, *Medicago sativa*; maize, MG, *Zea m ais* and a commercial concentrate, Conc) were used as substrates. The feedstuffs, after grinding to pass a 1 mm screen (Brabender Wiley mill, Brabender OHG Duisburg, Germany), were analysed in duplicate for dry matter; ether extract, ash, crude protein and crude fibre; acid detergent fibre (ADF) and acid detergent lignin (ADL) and amylase-treated neutral detergent fibre (NDF); using AOAC (2004) methods 934.01; 945.18; 973.18 and 2002.04, respectively. Chemical composition of samples used in the trial was reported in Table I.

Cumulative gas production was measured according to the IVGPT proposed by Theodorou et al. (1994). Caecal content and faeces of ostriches were used as *inocula*. For each substrate, 1.0032 ± 0.0012 g of sample (in triplicate per *inoculum*) was weighed in 120 ml serum flasks and 75 ml of anaerobic buffered modified (without tripticase-peptone) medium D (Theodorou, 1993) and 4 ml of reducing solution were added. Three flasks per *inoculum* were prepared without substrate and used as "blanks". The flasks were sealed with butyl rubber stoppers and aluminium crimp seals and incubated at 39°C until inoculation (around 12 hours).

Inocula collection and preparation

Caecal content and faeces were collected in the morning, in a specialised slaughter house, on four male ostriches raised on a commercial farm near Napoli (Italy). The average live weight of the animals was 94.2 ± 3.02 kg. From the fourth month to slaughter age (10 months) the ostriches were fed *ad libitum*, and the chemical-nutritional characteristics of the diet are reported in Table II. The chemical composition of the ostrich diet was determined according to the AOAC (2004) procedures indicated for the substrates. The content of crude energy was estimated using the equation proposed by Jentsch et al. (1963). The animals were fasted overnight before slaughter, but water was available.

Once the whole gastro-intestinal tract had been isolated, the caecal contents and faeces were collected immediately after dissection of caecum and cloaca, respectively, from the four ostriches and put each one into a pre-warmed thermos (around 39°C), filled to the brim in order to keep air content to a minimum. The samples were transported as soon as possible (about 1 hour) to our laboratories. In the laboratory, the caecal contents from four ostriches were pooled and 100 ml was diluted with 100 ml of the anaerobic modified medium D (without tripticase-peptone), stirred for 5 min and strained through six layers of gauze under CO_2 flow to maintain anaerobiosis. The solids retained on the gauze were mixed with another 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 gauze; the resulting liquid was combined with the first strained fluid and held at 39°C under CO_2 until use (final dilution 2:1 medium:caecal content).

In order to obtain an *inoculum* which was easy to introduce into the flasks, the faeces had to be diluted more than the caecal content. The fresh faeces from four ostriches were pooled and 100 g was added to 200 ml of anaerobic medium, stirred and strained through six layers of gauze. The solids retained on the gauze were then re-suspended in

Table I. Chemical composition of the substrates used in the trial (% DM).

	DM	Ash	CP	EE	NDF	ADF	ADL
DA	88.64	17.28	21.25	2.40	42.58	27.62	4.96
Conc[a]	89.66	12.47	28.93	5.68	25.31	9.27	0.59
AH	88.61	16.91	24.22	2.15	44.61	29.10	5.04
MG	91.93	1.38	7.57	3.56	16.10	4.31	0.55

DA, dehydrated alfalfa; Conc, concentrate; AH, alfalfa hay; MG, maize; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.
[a]Ingredients: Toasted soybean meal, wheat, sunflower meal, toasted soybean, wheat middling, corn gluten, di-calcium phosphate, calcium carbonate, soya oil, sodium bicarbonate, sodium chloride, magnesium oxide, DL-methionine, l-lysine.

Table II. Chemical-nutritional characteristics of ostrich diet (% DM).

	DM	Ash	CP	EE	NDF	ADF	ADL	CE, MJ/kg DM
Diet[a]	89.75	8.5	17.3	3.7	38.7	26.1	5.5	18.22

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.
[a]Ingredients: 40% DM dehydrated alfalfa meal, 35% DM concentrate (toasted soybean meal, wheat, sunflower meal, toasted soybean, wheat middling, corn gluten, di-calcium phosphate, calcium carbonate, soya oil, sodium bicarbonate, sodium chloride, magnesium oxide, and DL-methionine, l-lysine), 25% cereal mix.

200 ml of medium and homogenised by blending for 20 s. The homogenate was strained through six layers of gauze, mixed with the first strained solution and held at 39°C under CO_2 until use (final dilution 4:1 medium:faeces). The large dilution of the faeces was also required in order to obtain a better separation of the micro-organisms from the *digesta* (Omed et al., 2000).

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 *inoculum* 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 hours.

Gas measurements and analysis at the end of incubation

Gas production was recorded at 2, 4, 6, 8, 12, 14, 18, 22, 24, 28, 30, 35, 39, 43, 48, 52, 60, 64, 68, 72, 96 and 120 hours post-inoculation. Initial readings were taken at 2-hour intervals due to the rapid rate of gas production. The gas measurements were made using a manual 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 flasks, after stirring, returned to the incubator. At the end of incubation (120 hours), the flasks were placed at 4°C to terminate fermentation. The pH of each flask was recorded (Alessandrini Instrument glass electrode, Jenway, Dunmow, UK; model 3030) and two samples, each of about 10 ml of liquid, were collected and frozen prior to VFA and ammonia analysis. Dry matter digestibility of the substrates was estimated by filtering the residues through 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 the difference following ashing (5 hours at 550°C). Gas volumes obtained were related to the amount of incubated (organic matter cumulative volume, OMCV).

VFAs were determined, after centrifugation and dilution of the samples with oxalic acid (1:1 v/v) by the gas chromatography method (ThermoElectron mod. 8000 top, FUSED SILICA Gas chromatograph with OMEGAWAX 250 fused silica capillary column 30 m $\times$ 0.25 mm $\times$ 0.25 mm film thickness; analysis temperature, 125°C; flame ion detector, 185°C; carrier Helium 1.7 ml/min). Branched chain proportion (BCP), a valuable index of protein digestion, was determined as the sum of isobutyrate and isovalerate divided by the total VFA production.

Ammonia was determined according to the method described by Searle (1984). In short, the samples, after centrifugation at 1900 rpm for 10 min at room temperature (about 22°C), were diluted 10 times with water and, then, 1 ml of the product was deproteinised using 10% trichloro-acetic acid. Ammonia and phenol were oxidised by sodium hypochlorite in the presence of sodium nitroprusside to form a blue complex. The intensity was measured colorimetrically at a wavelength of 623 nm. Intensity of the blue colour is proportional to the concentration of the ammonia present in the sample.

Curve fitting and statistical analysis

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

$$G(t) = A/\left[1 + (B/t)^{c}\right]$$

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.

Groot's model was preferred since it was also used in previous trials by our research group. 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):

$$R\max = \left[A \times B^{\mathrm{C}} \times \mathrm{C} \times T\max^{(-\mathrm{C}-1)}\right] / \left[1 + \left(B^{\mathrm{C}}\right) \times T\max^{-\mathrm{C}}\right]$$

$$T\max = B \times \left[(\mathrm{C}-1)/(\mathrm{C}+1)^{1/\mathrm{C}}\right]$$

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

$$Y_{ijk} = \mu + S_i + I_j + \mathrm{SI}_{ij} + \varepsilon_{ijk}$$

where Y is the single observation; μ is the general mean; S is the substrate effect (I = DA, concentrate, alfalfa hay and maize); I is the *inocula* effect (j = caecal or faeces); SI is the interaction between the effects; and ε is the error.

The equations able to predict the fermentation characteristics of the caecal content from those of the faeces were studied by PROC REG (SAS, 2000).

Results

Caecal content and faeces characteristics

Table III reports the physico-chemical characteristics of caecal content and faeces used as a source of *inoculum*. The faeces showed higher pH (7.92 vs. 6.84), dry matter (29.68 vs. 10.02%), ash (10.21 vs. 3.92% DM), ammonia (7.86 vs. 7.30 mmol/l) and fibrous fractions (NDF; ADF and ADL) than caecal content. The other parameters (tVFA, acetate, propionate, butyrate, isobutyric, valerate and isovalerate acids) were higher for caecal content.

In vitro *fermentation characteristics*

As reported in Table IV, caecal *inoculum* showed a significant ($p < 0.01$) higher organic matter digestibility and gas production (OMCV, A) than FI (Table IV) The time required to produce A/2 (B) was significantly ($p < 0.01$) higher for FI.

Regarding the substrates, DA showed the lowest OMd (61.32%) and maize grain the highest (84.87%). Alfalfa hay showed OMd higher than DA; maize grain had the highest fermentation rate (10.86 ml/h) but the fermentation rate was lower than the other substrates (Tmax 26.82 hours).

The interaction between the effects was not statistically significant.

Table III. Physical and chemical characteristic of the two sources of inoculums.

	Caecal content	Faeces
Ph	6.84	7.92
Dry matter, %	10.02	29.68
Ash, % DM	3.92	10.21
Crude protein, % DM	12.88	11.08
Ether extract, % DM	2.27	1.27
NDF, % DM	26.06	39.13
ADF, % DM	14.92	22.37
ADL, % DM	3.48	6.12
Ammonia, mmol/l	7.30	7.86
Acetate, mmol/l	31.85	9.18
Propionate, mmol/l	8.20	3.33
Butyrate, mmol/l	3.21	0.78
Isobutyric, mmol/l	0.86	0.37
Valeric, mmol/l	0.40	0.14
Isovaleric, mmol/l	1.34	0.46
Total VFA, mmol/l	45.85	14.26

DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; VFA, volatile fatty acids.

End products of fermentation

After 120 hours of incubation (Table V), the FI showed a higher pH (6.82 vs. 6.71, $p < 0.01$) and a lower production of acetate, propionate and total VFA ($p < 0.01$) than CI. No significant differences were recorded for butyrate production.

Among substrates, MG showed the lowest pH (6.44) and AH the highest (6.93). The concentrate had the lowest production of acetate and the highest of propionate, while a very high production of butyrate and of total VFA was recorded for MG. Alfalfa hay showed a significantly higher ($p < 0.01$) organic matter digestibility after 120 hours of incubation than DA.

No significant interaction was recorded between the two tested effects.

Protein degradation

For almost all the parameters in Table VI, FI showed significantly ($p < 0.01$ or $p < 0.05$) lower values than CI. Just for ammonia, the values recorded for FI were significantly ($p < 0.01$) higher than CI. Among the substrates, maize had the lowest productions of isobutyric, isovalerate and ammonia as well as BCP value. The highest valerate production was recorded for DA.

Table IV. *In vitro* fermentation characteristics by inocula and substrates.

	OMd, %	OMCV, ml/g	*A*, ml/g	*R*max, ml/h	*T*max, h
		Inoculum effect			
CI	76.83^{A}	320.1^{A}	250.3^{A}	7.60	16.15^{B}
FI	72.79^{B}	225.2^{B}	229.3^{B}	7.07	17.01^{A}
		Substrate effect			
DA	61.32^{C}	260.9^{B}	205.2^{BC}	5.37^{C}	15.69^{B}
Conc	81.71^{A}	270.0^{B}	235.9^{B}	7.08^{B}	11.77^{C}
AH	71.35^{B}	338.5^{A}	195.6^{C}	6.06^{BC}	12.04^{BC}
MG	84.87^{A}	221.9^{C}	322.6^{A}	10.86^{A}	26.82^{A}
		Interaction inoculum × substrate			
IE	NS	NS	NS	NS	NS
MSE	3.25	256.1	98.38	1.57	3.09

OMd, organic matter degradation; OMCV, organic matter cumulative volume; *A*, potential gas production; *R*max, maximum fermentation rate; *T*max, time to reach *R*max; CI, caecum inoculum; FI, faecal inoculum; DA, dehydrated alfalfa; Conc, concentrate; AH, alfalfa hay; MG, maize; IE, interaction between the effects; NS, not significant; MSE, mean square error.
A, B, C = $p < 0.01$.

Also for these parameters, the interaction between the effects was not statistically significant.

Estimation of caecal fermentation characteristics from faeces

The graphics representing the estimation of caecal fermentation characteristics from faeces are reported in Figure 1. For pH, organic matter degradation, acetate and ammonia the *r* value were, respectively, 0.9999, 0.9913, 0.9962 ($p < 0.01$) and 0.9625 ($p < 0.05$). For the other parameters not reported as graphics, the *r* values, due to the low number of data, were lower than 0.8, and the statistical significance was not reached.

Table V. End product profile after 120 hours of incubation.

	pH	Acetate	Propionate	Butyrate	tVFA
			mmol/gOM		
		Inoculum effect			
CI	6.71^{B}	57.91^{A}	16.41^{A}	9.34	86.91^{A}
FI	6.82^{A}	53.20^{B}	12.44^{B}	9.09	77.26^{B}
		Substrate effect			
DA	6.83^{B}	52.13^{B}	10.71^{C}	4.17^{C}	70.37^{C}
Conc	6.83^{B}	41.26^{C}	20.11^{A}	8.52^{B}	72.74^{C}
AH	6.93^{A}	68.69^{A}	10.92^{C}	3.82^{C}	86.85^{B}
MG	6.44^{C}	60.13^{AB}	15.96^{B}	20.35^{A}	98.38^{A}
		Interaction inoculum × Substrate			
IE	NS	NS	NS	NS	NS
MSE	0.0007	2.50	1.20	0.91	5.52

VFA, volatile fatty acids; CI, caecum inoculum; FI, faecal inoculum; DA, dehydrated alfalfa; Conc, concentrate; AH, alfalfa hay; MG, maize; IE, interaction between the effects; NS, not significant; MSE, mean square error.
A, B, C = $p < 0.01$.

Table VI. Protein degradation products.

	Isobutyric mmol/gOM	Isovalerate mmol/gOM	BCP	Valerate mmol/gOM	NH3, mmol/l
		Inoculum effect			
CI	1.17^{A}	1.49^{A}	0.031^{a}	0.59^{A}	21.63^{B}
FI	1.01^{B}	1.10^{B}	0.027^{b}	0.42^{B}	24.20^{A}
		Substrate effect			
DA	1.21^{AB}	1.25^{B}	0.035^{AB}	0.90^{A}	24.23^{A}
Conc	1.16^{B}	1.59^{A}	0.038^{A}	0.10^{C}	26.85^{A}
AH	1.34^{A}	1.49^{A}	0.033^{B}	0.59^{B}	26.55^{A}
MG	0.66^{C}	0.85^{C}	0.015^{C}	0.43^{B}	14.03^{B}
		Interaction inoculum × Substrate			
IE	NS	NS	NS	NS	NS
MSE	0.02	0.04	64×10^{-5}	0.02	6.23

CI, caecum *inoculum*; FI, faecal *inoculum;* BCP, branched chain proportion, (isobutyric + isovalerate)/tVFA; NH3, ammonia; DA, dehydrated alfalfa; Conc, concentrate; AH, alfalfa hay; MG, maize; IE, interaction between the effects; NS, not significant; MSE, mean square error.
A, B, C = $p < 0.01$, a, b = $p < 0.05$.

Discussion

The higher percentage of OMd obtained with caecal *inoculum* indicates that caecal microflora have a higher fermentative on the tested substrates. The higher production of acetate and propionate recorded for CI (Table V) confirmed a more intense fermentation of structural (in particular, cellulose) and non-structural carbohydrates. In fact, it is known (Van Soest, 1993) that acetic acid production is from the fermentation of cellulolytic bacteria, while propionic acids are from non-structural carbohydrates fermentations. Moreover, during fermentations, mainly acetate and butyrate synthesis contribute to gas production. In fact, the fermentation of 1 mole of glucose to acetate results in the production of 1 mole of CO_2 and 1 mole of CH_4, and the fermentation to butyrate results in the production of 1.5 mole of CO_2 and 0.5 mole of CH_4. On the other hand, the fermentation of 1 mole of glucose to propionate does not result in a net production of CO_2 and requires a net input of reducing equivalents, resulting in a decrease in CH_4 production (Ungerfeld & Kohn, 2006). Thus, the significantly higher productions of acetate obtained with CI *inoculum* can justify the significantly higher gas production and strongly suggest a higher fermentation activity by microflora when using structural carbohydrates.

CI *inoculum* also showed a more intense fermentative activity in respect of protein contained of the tested feeds. This assertion comes from the observation of the significantly higher BCP value recorded for CI. Since isobutyrate, isovaleric and valeric acids are produced, respectively, from degradation

of the amino acids valine, leucine and proline (Van Soest, 1994), their higher production suggests higher protein degradation (Bovera et al., 2007). Also NH_3 is an end product of protein fermentation, but it is used from bacteria, in combination with carbon chains produced from carbohydrates fermentation to synthesise new amino acids for bacterial growth (Van Soest, 1994). NH_3 content is lower with CI. Thus, in the case of CI not only the fermentations of proteins and structural carbohydrates are more intensive but also there is, probably, a better synchronism in both carbohydrate and protein fermentation that allow bacteria to dispose of both carbon chains and NH_3 for their protein synthesis and thus for increasing bacterial biomass.

Our results partly agree with the findings of Bovera et al. (2007). The differences in *in vitro* fermentation characteristics of the two *inocula* could be due to differences in microflora composition and/or activity. Recent researches based on PCR (Matsui et al., 2010) identified the following *phyla* in microbial population of ostrich caecum: *Firmicutes* (50.9% of the total number of sequences), *Bacteroidetes* (39.4%), *Fibrobacteres* (6.5%), *Euryarchaeota* (1.9%), *Spirochaetes* (1.0%) and *Verrucomicrobia* (0.3%); so, approximately 90% of the sequences were affiliated with *Firmicutes* and *Bacteroidetes*. Five fibrolytic bacteria from ostrich faeces have been isolated and identified (van Gylswyk et al., 1998), including isolates having homology (97–99%) to *Propionibacterium acnes* and one with a 16S ribosomalDNA (rDNA) sequence having homology (97%) to *Ruminococcus flavefaciens*.

However, we have to consider that ostriches used in the trial fed the diet for a long period and their intestinal microflora is well adapted to the alfalfa structural carbohydrate digestion than other potential fibre source.

The fermentation characteristics of the two *inocula* are quite different but the interaction between substrate and *inoculum* is not significant, suggesting that the *inocula*, independently from fermented substrate, had a similar trend.

Scant and conflicting data are available in literature on the caecal content characteristics of ostriches. Józefiak et al. (2004) reported, in adult ostriches, caecal pH of 7 and total VFA content of 160 mmol/gOM. Hongo et al. (2006), studying the VFA production in the whole gastro-intestinal tract of adult ostriches, observed the highest production of VFA (25 mmol/gOM) in the caecum. The pH values recorded in our trial agree with Józefiak et al. (2004), while total VFA content is lower than those recorded by Józefiak et al. (2004) and higher than the values of Hongo et al. (2006). However, it is clear that pH as well as fatty acid production in the caecum is strongly related to animal age and nutrition.

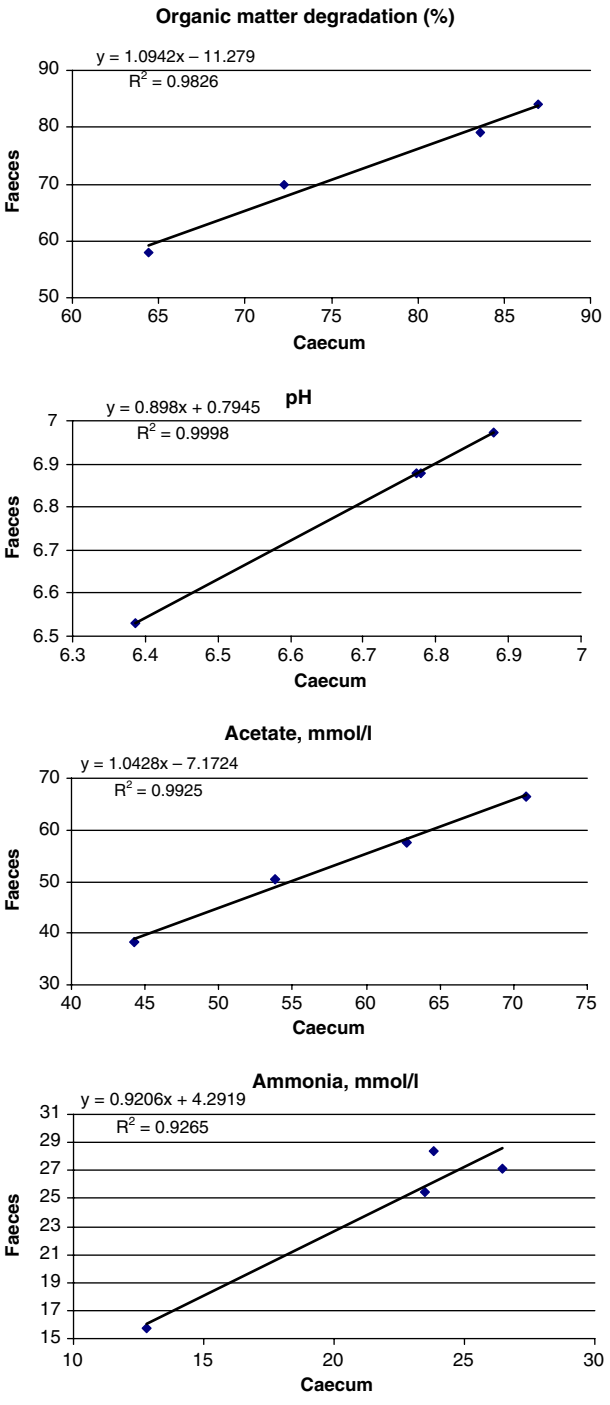

Figure 1. Regression equations to estimate some caecal fermentation parameters from faeces.

Chemical characteristics of caecal content and faeces of ostriches can be compared to those of other species of "hindgut fermenters" such as rabbits. Bovera et al. (2006) found a pH of 6.3 and 7.9, respectively, for caecal content and hard faeces of rabbits at slaughter (77 days), 12 hours fasted. In the same animals, VFA content was 59.0 and 1.9

mmol/l, respectively, for caecal content and hard faeces.

Substrates, according to their chemical composition, showed significant differences in terms of fermentation characteristics. For example maize, rich in starch, produced the lowest pH at the end of fermentations and, due to the low protein content, also the BCP value was the lowest. The alfalfa hay, as also observed from chemical composition (Table I) resulted in a very good quality (in particular higher crude protein content), higher than the DA. In fact, not only the organic matter degradation was significantly higher than DA, but also acetate production was significantly higher. However, no differences were recorded between AH and DA for BCP and ammonia production.

The regression equations estimated for the tested parameters (Figure 1) showed a good relation between the two *inocula* for some fermentation parameters. In this trial a low number of data were used to obtain the regression equations and, as a consequence, to reach a definitive conclusion it will be necessary to test a great number of samples. However, on the basis of the reported results it seems possible to estimate the fermentation characteristics of caecal content from that of faeces and, as a consequence, to use an easy *in vitro* technique to evaluate as the feeds are fermented and, indirectly, digested from the ostriches. Similar results were also obtained by Bovera et al. (2007).

Conclusions

Our results indicate that faeces may represent a suitable alternative to caecal fluid in order to estimate *in vitro* caecal fermentation characteristics of ostriches. There are several differences between the *inocula* in terms of substrate fermentation: caecal *inoculum* is more active in structural and non-structural carbohydrates fermentation as well as in protein degradation than faecal *inoculum*. However, it was possible to calculate significant equations in order to estimate caecal fermentation parameters from that of faeces. This is especially interesting for organic matter degradation, given the lack of available data for ostriches.

Undoubtedly, the accuracy of the equations could be improved by increasing the number of tested diets or feedstuffs but, in our opinion, the use of faeces as an alternative *inoculum* to caecal content could significantly contribute to improving our knowledge of ostrich nutrition.

References

Aiple, K. P., Steingass, H., & Menke, K. H. (1992). Suitability of a buffered faecal suspension as the inoculum in the Hohenheim gas test 1. Modification of the method and its ability in the prediction of organic matter digestibility and metabolisable energy content of ruminant feeds compared with rumen fluid as inoculum. *Journal of Animal Physiology and Animal Nutrition*, *67*, 57–66.

Association of Official Analytical Chemists (2004). *Official Methods of Analysis*, 18th ed., Vol. 2. (Arlington, VA, USA: AOAC).

Bauer, E., Williams, B. A., Voigt, C., Mosenthin, R., & Versteng Martin, W. A. (2001). Microbial activities of faeces from unweaned pigs, in relation to selected fermentable carbohydrates. *Animal Science*, *73*, 313–322.

Bindelle, J., Buldgen, A., Lambotte, D., Wavreille, J., & Leterme, P. (2007). Effect of pig faecal donor and of pig diet composition on in vitro fermentation of sugar beet pulp. *Animal Feed Science and Technology*, *132*, 212–226.

Bovera, F., Calabro, S., Cutrignelli, M. I., Infascelli, F., Piccolo, G., Nizza, S., Tudisco, R., & Nizza, A. (2008). Prediction of rabbit caecal fermentation characteristics from faeces by in vitro gas production technique: Roughages. *Journal of Animal Physiology Animal Nutrition*, *92*, 260–271.

Bovera, F., D'Urso, S., Calabrò, S., Tudisco, R., Di Meo, C., & Nizza, A. (2007). Use of faeces as alternative inoculum to caecal content to study the in vitro feed digestibility in domesticated ostriches (Struthio camelus var. domesticus). *British Poultry Science*, *48*, 354–362.

Bovera, F., D'Urso, S., Di Meo, C., Piccolo, G., Calabrò, S., & Nizza, A. (2006). Comparison of rabbit caecal content and rabbit hard faeces as source of inoculum for the in vitro gas production technique. *Asian-Australasian Journal of Animal Sciences*, *19*, 1649–1657.

Bovera, F., D'Urso, S., Di Meo, C., Tudisco, R., & Nizza, A. (2009). A model to assess the use of caecal and faecal inocula to study fermentability of nutrients in rabbit. *Journal of Animal Physiology and Animal Nutrition*, *93*, 147–156.

Calabrò, S., Monello, G., Piccolo, V., Bovera, F., Infascelli, F., Tudisco, R., & Cutrignelli, M. I. (2008). Rumen fermentation and degradability in buffalo and cattle using the in vitro gas production technique. *Journal of Animal Physiology and Animal Nutrition*, *92*, 356–362.

Calabrò, S., Tudisco, R., Pellikaan, W., Passaro, A., Cutrignelli, M. I., Bovera, F. and Infascelli, F. (2007) *Influence of carbohydrate-rich foodstuffs on intestinal microbial activity of adult pigs*. 11th Congress of the European Society of Veterinary and Comparative Nutrition, November 1st–3rd, Leipzig, Germany, 61.

Cilliers, S. C., Hayes, J. P., Chwalibog, A., Du Preez, J. J., & Sales, J. (1997). A comparative study between mature ostriches (Struthio camelus) and adult cockerels with respect to true and apparent metabolisable energy values for maize, barley, oats and triticale. *British Poultry Science*, *38*, 96–100.

Cone, J. W., Van Gelder, A. H., & Bachmann, H. (2002). Influence of inoculum source on gas production profiles. *Animal Feed Science and Technology*, *99*, 221–231.

Duke, G. E., Degen, A. A., & Reynhout, J. K. (1995). Movement of urine in the lower colon and cloaca of ostriches. *Condor*, *97*, 165–173.

Getachew, G., Blümmel, M., Makkar, H. P. S., & Becker, K. (1998). In vitro gas measuring techniques for assessment of

nutritional quality of feeds: A review. *Animal Feed Science Technology*, *72*, 261–281.

Groot, J. C. J., Cone, J. W., Williams, B. A., Debrasques, F. M. A., & Lantinga, E. A. (1996). Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feed. *Animal Feed Science and Technology*, *64*, 77–89.

Harris, D. M., Barlet, A., & Chamberlain, A. T. (1995). The use of dairy cow faeces rather than rumen liquor in the gas pressure transducer technique for assessing digestion kinetics in vitro. *Animal Science*, *60*, 541–543.

Hongo, A., Ishii, Y., Suzuta, H., Enkhee, D., Toukura, Y., Hanada, M., Hidaka, S., & Miyoshi, S. (2006). Position and rate of intestinal fermentation in adult ostrich evaluated by volatile fatty acid. *Research Bulletin of Obihiro University*, *27*, 93–97.

Jentsch, W., Scheimann, R., Hoffmann, L., & Nehhering, K. (1963). Die energetische verwetung der Futterstoffe. 2 Die energetische verwetung der Kraftfutterstoffe durch Kaninchen. *Archive Tierernahr*, *13*, 133–145.

Józefiak, D., Rutkowski, A., & Martin, S. A. (2004). Carbohydrate fermentation in the avian caeca: A review. *Animal Feed Science Technology*, *113*, 1–15.

Lan, Y., Williams, B. A., Verstegen, M. W. A., Patterson, R., & Tamminga, S. (2007). Soy oligosaccharides in vitro fermentation characteristics and its effect on caecal microorganisms of young broiler chickens. *Animal Feed Science Technology*, *133*, 286–297.

Matsui, H., Kato, Y., Chikaraishi, T., Moritani, M., Ban-Tokuda, T., & Wakita, M. (2010). Microbial diversity in ostrich ceca as revealed by 16S ribosomal RNA gene clone library and detection of novel Fibrobacter species. *Anaerobe*, *16*, 83–93.

Mauricio, R. M., Owen, E., Mould, F. L., Givens, I., Theodorou, M. K., France, J., Davies, D. R., & Dhanoa, M. S. (2001). Comparison of bovine rumen liquor and bovine faeces as inoculum for an in vitro gas production technique for evaluating forages. *Animal Feed Science Technology*, *89*, 33–48.

Menke, K. H. & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development*, *28*, 7–12.

Murray, J. M. D., Longland, A., & Moore-Colyer, M. (2006). In vitro fermentation of different ratios of high temperature dried Lucerne and sugar beet pulp incubated with an equine faecal inoculum. *Animal Feed Science Technology*, *129*, 89–98.

Musara, C., Chamunorwa, J. P., Holtug, K., & Skadhauge, E. (2002). Water absorption in relation to fermentation in the colon of the ostrich (*Struthio camelus*). *Onderstepoort Journal of Veterinary Research*, *69*, 315–320.

Omed, H. M., Lovett, D. K., & Axford, R. F. E. (2000). Faeces as a source of microbial enzymes for estimating digestibility. In D.I. Givens & E. Owen (eds.) *Forage Evaluation in Ruminant Nutrition*. New York: CABI Publishing.

SAS, (2000). *SAS/STAT® Software: Changes and Enhancements through Release 8.1*. Cary, NC: SAS Institute Inc.

Searle, P. L. (1984). The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. *Analyst*, *109*, 549–568.

Stanco, G., Di Meo, C., Calebrò, S., & Nizza, A. (2003). Prediction of nutritive value of diets for rabbits using an *in vitro* gas production techinque. *World Rabbit Science*, *11*, 199–210.

Theodorou, M. K. (1993). A new laboratory procedure for determining the fermentation kinetics of ruminants feeds. *Ciencia y Investigacion Agraria*, *20*, 332–344.

Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., & France, J. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, *48*, 185–197.

Ungerfeld, E. M. & Kohn, R. A. (2006). The role of thermodynamics in the control of ruminal fermentation. In K. Sejrsen, T. Hvelplund, & M.O. Nielsen (eds.) *Ruminant Physiology*, pp. 55–85. Wageningen Academic.

van Gylswyk, K. O., Russouw, T., & James, M. D. (1998). Bacterial cellulose degradation in the ostrich digestive tract. In K. Ohmiya, K. Hayashi, K. Sakka, Y. Kobayashi, S. Karita, & T. Kimura (eds.) *Genetics, Biochemistry and Ecology of Cellulose Degradation*, pp. 602–610. Tokyo, Japan: Uni Publishers.

Van Soest, P. J. (1993). Cell wall matrix interactions and degradation – Session synopsis. In H. G. Jung, D. R. Buxton, R. D. Hatfield, & J. Ralph (eds.) *Forage Cell Wall Structure and Digestibility*, p. 377. Madison, WI: ASA-CSSA-SSSA.

Van Soest, P. J. (1994). *Nutritional Ecology of the Ruminant* (2nd ed., pp. 354–370). Ithaca, NY: Comstock.