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Use of faeces as an alternative inoculum to caecal content to study *in vitro* feed digestibility in domesticated ostriches (*Struthio camelus* var. *domesticus*)

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Abstract 1. In order to find an alternative source of inoculum to caecal content for studying the *in vitro* feed digestibility in domesticated ostriches (*Struthio camelus* var. *domesticus*), caecal content and faeces of 4 male birds were used as inocula for an *in vitro* gas production trial.

2. About 1 g of each of 5 substrates (maize silage, CS; alfalfa hay, AH; barley, BG; soybean meal, SM; beet pulp, BP) was weighed, in quadruplicate per inoculum, in 120 ml flasks; 75 ml of anaerobic medium and 4 ml of reducing solution were added and flasks were kept at 39°C. Caecal content and faeces were diluted respectively 1 : 2 (CI) and 1 : 4 (FI) with an anaerobic medium and were injected into the respective flasks (10 ml).

3. Gas production was recorded 22 times up to 120 h of incubation and fermentation characteristics (for instance, degraded organic matter, OMd; potential gas production, *A*; maximum fermentation rate, R_{max}; time at which it is reached, T_{max}; pH; volatile fatty acid, VFA; ammonia) were studied for each inoculum and substrate.

4. CI and FI showed significant differences in T_{max} (16·37 *vs* 18·47 h, respectively), propionic (16·47 *vs* 12·07 mmoles/l) and butyric acid (6·50 *vs* 7·98 mmoles/l) and ammonia concentration (17·18 *vs* 19·95 mmoles/l). The substrates, according to their chemical composition, showed different fermentation characteristics. However, the regression equations able to estimate some fermentation characteristics of the caecum from those of faeces were statistically significant and showed R^2-values ranging from 0·87 to 0·99.

5. The differences in fermentation pathways of the two inocula did not appear to influence the rate and extent of OM digestion. Faecal fermentation predicted rates and extent of OM digestion by caecal fermentation in ostriches; consequently, the faeces could be considered as an alternative to caecal content to study feed digestibility in the species, although there is a need to undertake further research.

INTRODUCTION

To obtain a useful feed value, it is necessary to determine digestibility specifically 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.

Feed digestibility of ostriches *in vivo* has been determined using an *ingesta-excreta* balance method (Cilliers *et al*., 1997). 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 (1963) method, concluded that the method, commonly used for ruminants, needs to be modified to suit ostriches.

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

	DM (%)	CP	CF	EE	Ash	NDF	ADF	ADL
CS	33·93	8·26	21·51	2·15	5·95	37·26	29·24	4·12
AH	90·00	13·10	29·04	1·31	7·53	47·55	44·48	9·82
BG	87·20	9·62	6·77	1·49	3·29	22·09	8·28	1·60
SM	87·49	43·19	1·21	3·13	6·53	14·75	9·29	0·81
BP	88·13	9·32	20·18	0·95	5·15	51·80	30·86	1·89

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

Since the 1990s there has been increasing interest in the *in vitro* technique which measures gas production both to study rumen fermentation kinetics (Blümmel and Becker, 1997; Getachew *et al*., 1998; Calabrò *et al*., 2002) and to estimate the *in vivo* digestibility of ruminant feeds (Menke and Steingass, 1988; Blümmel and Ørskov, 1993). Given the valid results obtained and relatively straightforward low-cost trials, the *in vitro* gas production technique (IVGPT) was recently used also in other species such as rabbits (Calabrò *et al*., 1999; Gazaneo *et al*., 2003; Stanco *et al*., 2003) and ostriches (Bovera *et al*., 2006) 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 (CO_2, CH_4 and traces of H_2) and volatile fatty acids (acetate, propionate, butyrate); gas production can, therefore, 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 ostriches the sampling of caecal content to use as inoculum for gas tests necessitates slaughter of the animal. However, in both cases, there are a number of ethical considerations regarding these techniques with respect to animal welfare. It is thus necessary to find an alternative source of inoculum for IVGPT. To this effect, a number of studies (El Shaer *et al*., 1987; Akhter *et al*., 1995; O'Donovan, 1995) recently reviewed by Omed *et al*. (2000) showed that faeces can represent a valid alternative source of inoculum to estimate *in vitro* digestibility as proposed by Tilley and Terry (1963).

The aim of our research was to compare the fermentative activity of caecal content and faeces of ostriches, when used as a source of inoculum in an IVGPT trial conducted on 5 feedstuffs commonly used as ingredients for ostrich diets.

MATERIAL AND METHODS

Substrate preparation

Five feeds (maize silage, CS, *Zea mais*; alfalfa hay, AH, *Medicago sativa*; barley, BG, *Hordeum vulgare*; soybean meal, SM, *Glycine max*; dehydrated beet pulp, DBP, *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 (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, $1{\cdot}0048 \pm 0{\cdot}0015$ 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 flasks per inoculum were prepared without substrate and were used as 'blanks'. The flasks 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 done in the morning in a specialised slaughterhouse on 4 male ostriches raised on a commercial farm in Naples (Italy), weighing on average $95{\cdot}6 \pm 3{\cdot}12$ kg. From the 4th to 10th month of age (the latter being the slaughter date) the ostriches were fed *ad libitum* on a diet consisting of dehydrated alfalfa (40% dry matter (DM)), commercial concentrate (35%) and a cereal mix (25%). From the night before slaughter, the animals were fasted, but water was available. Once the whole gastrointestinal tract had been isolated, the caecal content and faeces were collected and put into a pre-warmed vacuum flask, filled to the brim in order to keep air content to a minimum. After sampling, the material was transported as soon as possible (about one hour) to our department laboratories.

In the laboratory, 100 ml of pooled caecal content were diluted with 100 ml of anaerobic medium, stirred for 5 min and strained through

6 layers of gauze 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 6 layers of gauze; 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).

In order to obtain an inoculum which was easy to introduce into the flasks, the faeces had to be diluted more than caecal content. Fresh faeces (100 g) were added to 200 ml of anaerobic medium, stirred and strained through 6 layers of gauze. The remaining solids were then re-suspended in 200 ml of medium and homogenised by blending for 20 s. The homogenate was strained through 6 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). Considerable dilution of the faeces was also required in order to better separate the micro-organisms from the digesta (Omed *et al.*, 2000).

The time taken to prepare 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 displaced gas was allowed to escape and after inoculation the flasks were placed in an incubator at 39°C for 120 h.

Gas measurements and analysis at the end of incubation

Gas production was recorded at the following intervals post-inoculation : 2, 4, 6, 9, 12, 14, 16, 19, 21, 24, 27, 33, 36, 40, 44, 48, 52, 60, 68, 72, 96 and 120 h. Initial readings were taken at 2 h intervals because of 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 flasks, after stirring, were returned to the incubator. At the end of incubation (120 h), the flasks were cooled to 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 volatile fatty acid (VFA) and ammonia analysis. Substrate digestibility was estimated by filtering the residues using pre-weighed sintered glass crucibles (Scott Duran, porosity 2) under vacuum. Residual 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 (organic matter cumulative volume, OMCV) and degraded (yield of organic matter, YOM) organic matter.

After centrifugation and dilution of the samples with oxalic acid (1 : 1 v/v), the VFAs were analysed by the gas-chromatography method (ThermoElectron mod. 8000top, Fused Silica Gaschromatograph with OMEGAWAX 250 fused silica capillary column 30 m × 0·25 mm × 0·25 mm film thickness; analysis temperature 125°C; flame ion detector 185°C; carrier helium 1·7 ml/min).

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% trichloroacetic 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 is proportional to the concentration of 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):

$$G(t) = \frac{A}{[1 + (B/t)^C]}$$

where G (ml/g OM) is the amount of gas produced per g 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):

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

$$T_{max} = B \times \frac{C - 1}{(C + 1)^{1/C}}.$$

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

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

Table 2. *In vitro fermentation characteristics by inocula and substrates*

	OMd (%)	OMCV (ml/g)	YOM (ml/g)	A(ml/g)	B (h)	R_{max} (ml/h)	T_{max} (h)
Inoculum effect							
CI ($n=20$)	73·72	273·61	375·43	239·84	$26·94^{b}$	6·41	$16·37^{B}$
FI ($n=20$)	73·13	270·16	374·40	234·23	$27·86^{a}$	6·58	$18·47^{A}$
Substrate effect							
CS ($n=8$)	$61·94^{C}$	$245·06^{B}$	$396·97^{A}$	$226·47^{B}$	$28·04^{B}$	$5·27^{C}$	$15·50^{C}$
AH ($n=8$)	$55·02^{D}$	$217·34^{C}$	$395·07^{A}$	$186·17^{C}$	$26·63^{B}$	$4·49^{C}$	$14·65^{C}$
BG ($n=8$)	$81·34^{B}$	$327·31^{A}$	$402·77^{A}$	$275·87^{A}$	$22·75^{C}$	$10·04^{A}$	$18·18^{B}$
SM ($n=8$)	$88·26^{A}$	$253·69^{B}$	$287·39^{B}$	$220·86^{B}$	$27·38^{B}$	$5·04^{C}$	$13·29^{C}$
BP ($n=8$)	$80·57^{B}$	$316·04^{A}$	$392·37^{A}$	$275·82^{A}$	$32·19^{A}$	$7·24^{B}$	$25·48^{A}$
Significance							
I	NS	NS	NS	NS	*	NS	**
S	**	**	**	**	**	**	**
I × S	NS	NS	NS	NS	**	NS	**
MSE	15·69	267·41	329·83	163·37	1·99	0·49	2·39

CI = caecum inoculum; FI = faecal inoculum; CS = corn silage; AH = alfalfa hay; BG = barley grain; SM = soybean meal; BP = beet pulp; 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; R_{max} = maximum fermentation rate; T_{max} = time at which R_{max} is reached; I = inoculum effect; S = substrate effect; I × S = interaction between the effects.A, B, C, D and ** = $P<0·01$; a, b and * = $P<0·05$; NS=not significant; MSE = mean square error.

where Y is the single observation; μ is the general mean; S is the substrate effect (i = alfalfa hay, corn silage, soybean meal, beet pulp, barley); I is the inocula effect (j = caecum or faeces); SI is the interaction between the effects; and ε is the error. The equations to predict the fermentation characteristics of the caecal content from those of the faeces were studied by PROC REG (SAS, 2000).

RESULTS

In terms of fermentation characteristics by inoculum and substrate (Table 2) there were few major statistical differences between inocula. Only B and T_{max} values were significantly higher with FI than CI (B: 26·9 *vs* 27·9 h, respectively, for CI and FI, $P<0·05$; T_{max}: 16·4 *vs* 18·5 h, respectively, $P<0·01$).

As regards chemical composition (Table 1), major significant differences ($P<0·01$) were recorded among feeds. Interestingly, as a function of organic matter digestibility, the feeds can be classified as follows: SM > BG > BP > CS > AH while according to the gas produced per g of organic matter incubated (OMCV) as: BG > BP > SM > CS > AH. Finally, according to R_{max} and T_{max}, the feed classification was, respectively: BG > BP > CS > SM > AH and BP > BG > CS > AH > SM. The interaction was significant ($P<0·01$) for B and T_{max}.

To better understand the behaviour of the two inocula, Figures 1 and 2 report the trends in gas production and fermentation rate by substrate and inoculum, respectively.

The end product profile recorded after 120 h of incubation is shown in Table 3 along with the sum of acetate and butyrate related to propionate production [(A + B)/P]. CI produces greater concentrations of propionate (16·5 *vs* 12·1 mmoles/l, respectively, for CI and FI, $P<0·01$) and lower butyrate (6·5 *vs* 8·0 mmoles/l, respectively, $P<0·05$). Acetate and total VFA production were no different between inocula though FI showed a higher level of acetate and lower tVFA than CI.

Regarding the substrate effect, barley induced significantly higher productions of acetate, butyrate and tVFA and a higher (acetate + butyrate)/propionate ratio [(A + B)/P]. Beet pulp, though rich in structural carbohydrates, showed the highest production of propionate and hence the lowest (A + B)/P ratio. The production of the most important VFAs (acetate, propionate and butyrate) and total VFA production are also represented in Figure 3 by inoculum and substrate.

Table 4 shows the products of protein degradation and the branched chain proportion [BCP = (isobutyric + isovaleric)/tVFA] as a function of inocula and substrates. Comparison between inocula showed a higher BCP with CI (0·030 *vs* 0·027, $P<0·05$) and a higher ammonia production with FI (19·65 *vs* 17·18 mmoles/l, $P<0·01$). As expected, soybean meal showed significantly higher values ($P<0·01$) for all the parameters reported in Table 4. Finally, Table 5 reports the regression equations able to estimate some fermentation characteristics of the caecal content from those of the faeces.

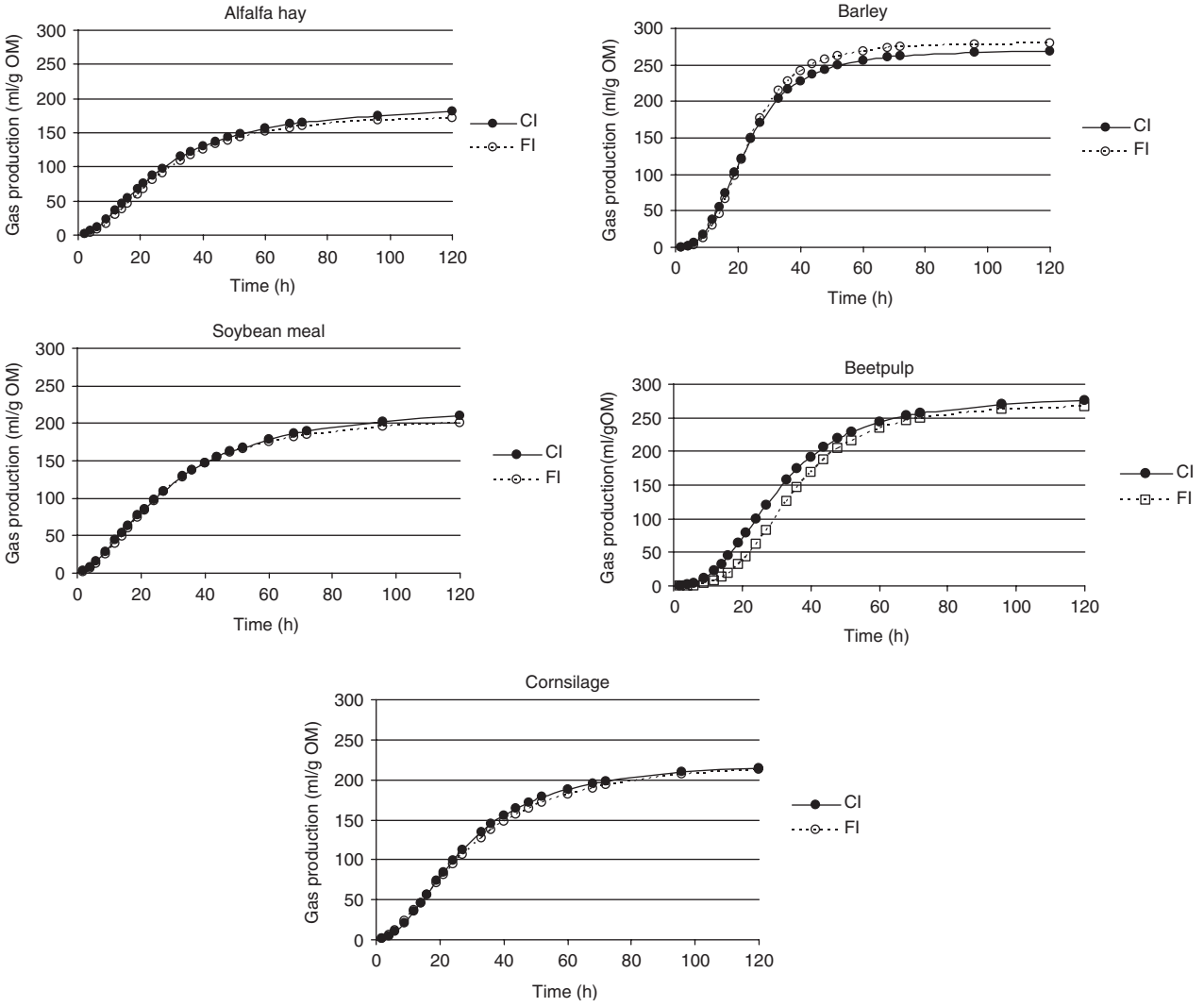

Figure 1. *Trend of gas production by inocula and substrates. CI = caecal inoculum; FI = faecal inoculum.*

DISCUSSION

The time required to reach the maximum rate of fermentation is higher for faecal inoculum, and hence the fermentation process is generally slower with FI. Looking at the trend of gas production for each feed, it emerges that the curves produced by the two inocula are similar in shape. In particular, the two inocula generated gas production curves almost superimposable with alfalfa hay, soybean meal and corn silage, while FI showed higher gas production after the 24th h of incubation with the barley and lower gas production at each time with beet pulp.

In the case of fermentation rate the two inocula also showed some differences in function of the fermented substrate. In particular, the FI showed a lower fermentation rate when the structural carbohydrates are more complex (alfalfa hay, corn silage). By contrast, CI showed a lower fermentation rate with the concentrates, in particular with the barley. Moreover, the two inocula showed similar fermentation rate curves for beet pulp, but the lag phases were longer with FI.

Since acetate and butyrate come from fermentation of structural carbohydrates and propionate from that of non-structural carbohydrates, the results reported in Table 3 could appear to conflict with those shown in Figures 1 and 2. However, close examination of Figure 3, which reports the graphs of the major VFA and tVFA production by inoculum and substrates, shows the particular behaviour of barley. In fact, while for the other substrates the caecal inoculum almost always induced higher VFA production, for barley the levels of acetate, butyrate and propionate were higher with the faecal inoculum (acetate: 46·2 *vs* 67·3 mmoles/l, respectively, for CI and FI; butyrate: 10·1 *vs* 12·7 mmoles/l; tVFA: 72·1 *vs* 95·4 mmoles/l) and the production of propionate was in practice superimposable. Moreover, it is worth noting that the amount of acetate produced by faecal inoculum with barley was the greatest of all. Therefore, also in this case, better use of the concentrates with FI is

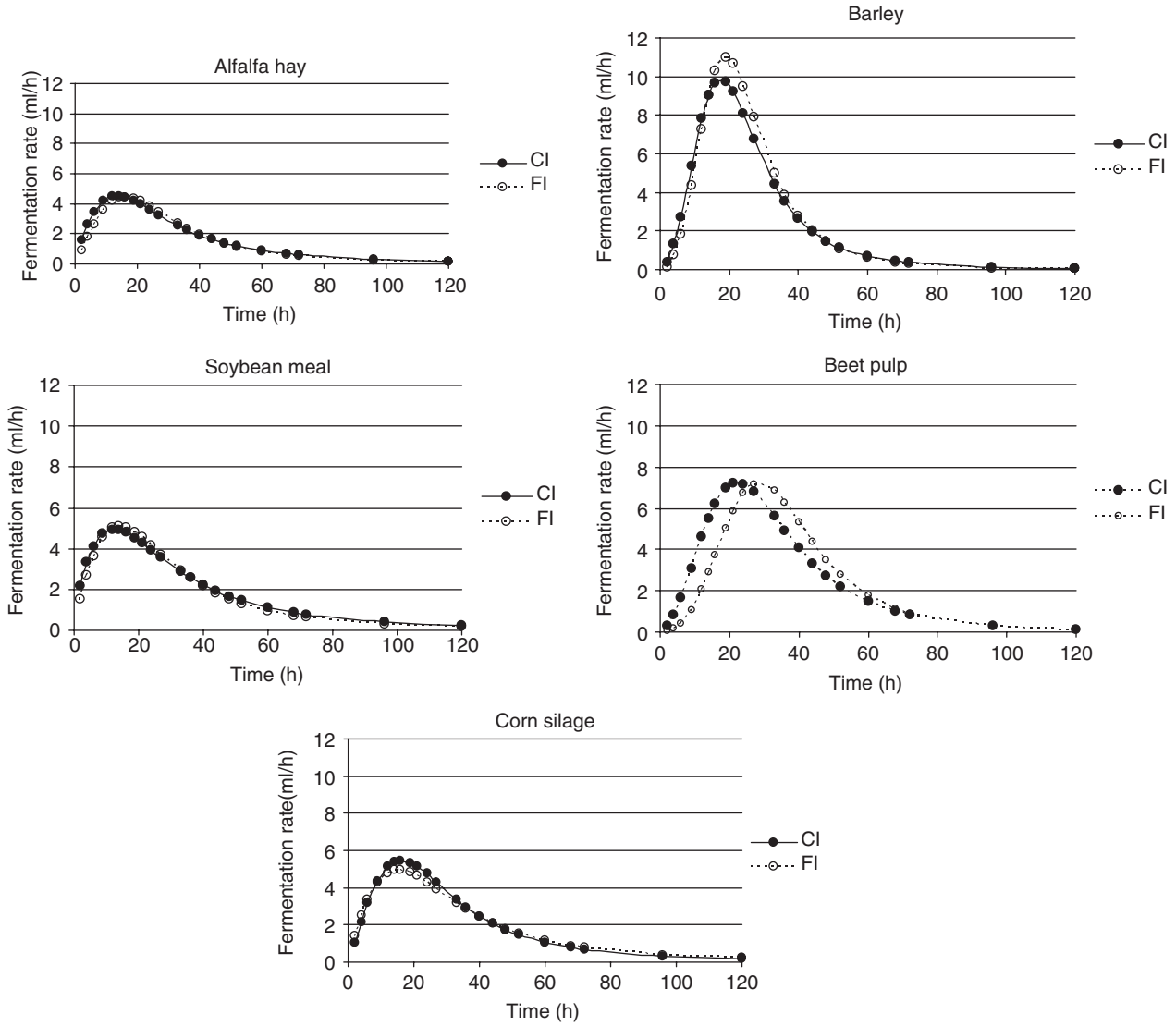

Figure 2. *Trend of the fermentation rate by inocula and substrates. CI = caecal inoculum; FI = faecal inoculum.*

Table 3. *End product profile after 120 h of incubation*

	pH	Acetate (mmoles/l)	Propionate (mmoles/l)	Butyrate (mmoles/l)	TVFA (mmoles/l)	(A + B)/P
Inoculum effect						
CI ($n = 20$)	6·49	40·40	$16·47^{A}$	$6·50^{b}$	71·09	$3·31^{B}$
FI ($n = 20$)	6·51	44·26	$12·07^{B}$	$7·98^{a}$	66·45	$5·01^{A}$
Substrate effect						
CS ($n = 8$)	$6·46^{B}$	$30·39^{C}$	$9·10^{C}$	$9·75^{AB}$	$50·36^{C}$	$5v41^{A}$
AH ($n = 8$)	$6·68^{A}$	$46·34^{AB}$	$11·84^{BC}$	$3·86^{C}$	$64·55^{BC}$	$4·40^{AB}$
BG ($n = 8$)	$6·36^{C}$	$56·79^{A}$	$12·89^{BC}$	$11·60^{A}$	$83·78^{A}$	$5·34^{A}$
SM ($n = 8$)	$6·66^{A}$	$42·45^{BC}$	$16·03^{B}$	$6·60^{BC}$	$69·89^{AB}$	$3·22^{BC}$
BP ($n = 8$)	$6·38^{C}$	$48·19^{AB}$	$21·50^{A}$	$4·36^{C}$	$75·29^{AB}$	$2·44^{C}$
Significance						
I	NS	NS	**	*	NS	**
S	**	**	**	**	**	**
I × S	*	**	*	**	**	**
MSE	2·96	60·93	6·76	3·57	114·94	0·73

CI = caecum inoculum; FI = faecal inoculum; CS = corn silage; AH = alfalfa hay; BG = barley grain; SM = soybean meal; BP = beet pulp; tVFA = total volatile fatty acids; (A + B)/P = (acetate + butyrate)/propionate; I = inoculum effect; S = substrate effect; I × S = interaction between the effects.A, B, C, D and ** = $P < 0·01$; a, b and * = $P < 0·05$; NS = not significant; MSE = mean square error.

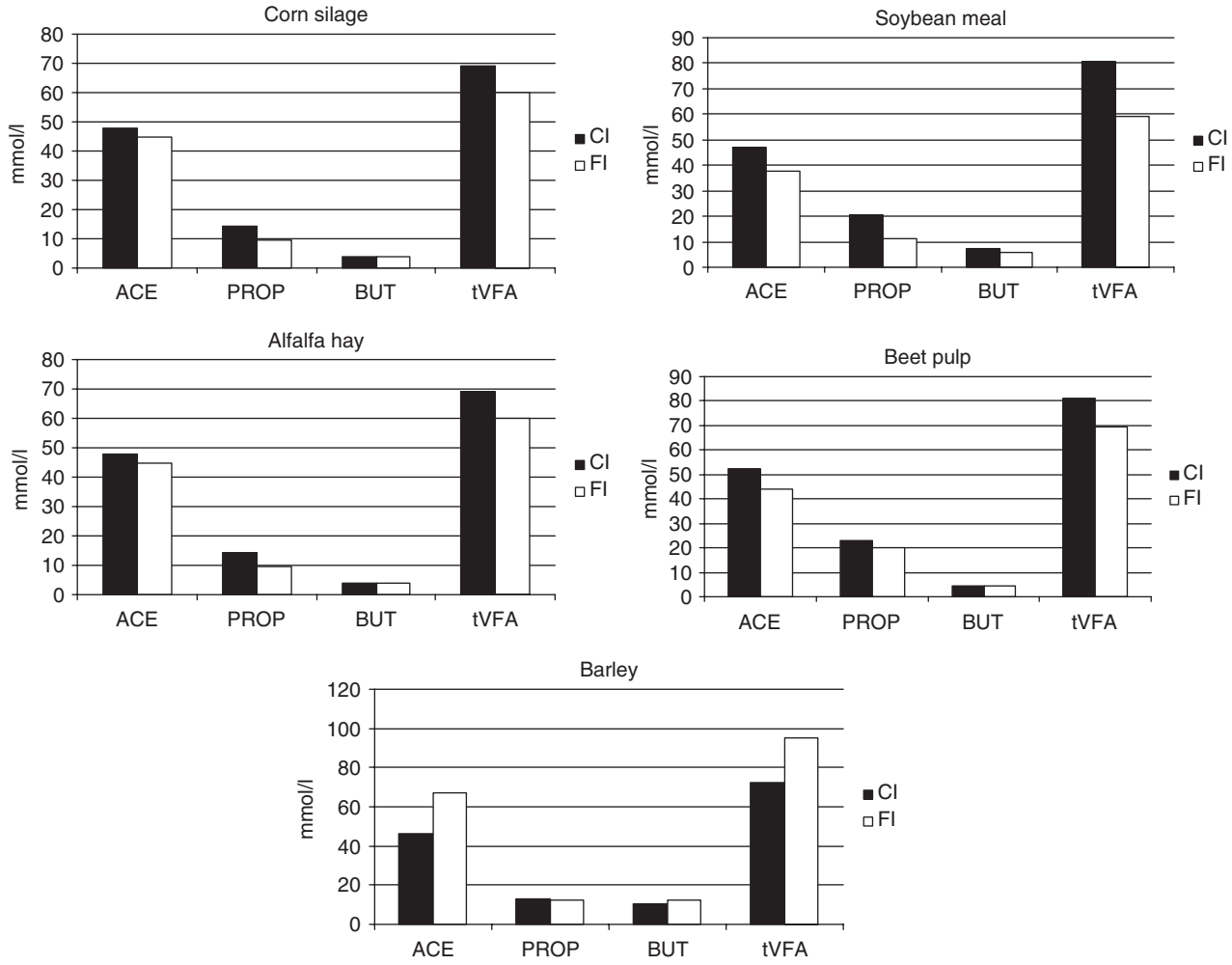

Figure 3. *Volatile fatty acid production by inocula and substrates. ACE = acetate; PROP = propionate; BUT = butyrate; tVFA = total volatile fatty acids; CI = caecal inoculum; FI = faecal inoculum.*

Table 4. *Protein degradation products*

	Isobutyric (mmoles/l)	Isovaleric (mmoles/l)	BCP	Valeric (mmoles/l)	NH_3 (mmoles/l)
Inoculum effect					
CI ($n = 20$)	$0{\cdot}97^{A}$	$1{\cdot}27^{A}$	$0{\cdot}030^{a}$	$0{\cdot}50^{a}$	$17{\cdot}18^{B}$
FI ($n = 20$)	$0{\cdot}79^{B}$	$1{\cdot}00^{B}$	$0{\cdot}027^{b}$	$0{\cdot}35^{b}$	$19{\cdot}65^{A}$
Substrate effect					
CS ($n = 8$)	$0{\cdot}42^{D}$	$0{\cdot}42^{D}$	$0{\cdot}017^{C}$	$0{\cdot}27^{B}$	$11{\cdot}42^{C}$
AH ($n = 8$)	$0{\cdot}77^{BC}$	$0{\cdot}89^{BC}$	$0{\cdot}026^{B}$	$0{\cdot}85^{A}$	$16{\cdot}40^{B}$
BG ($n = 8$)	$0{\cdot}98^{B}$	$1{\cdot}15^{B}$	$0{\cdot}025^{B}$	$0{\cdot}37^{B}$	$15{\cdot}85^{B}$
SM ($n = 8$)	$1{\cdot}68^{A}$	$2{\cdot}71^{A}$	$0{\cdot}062^{A}$	$0{\cdot}42^{B}$	$38{\cdot}53^{A}$
BP ($n = 8$)	$0{\cdot}53^{CD}$	$0{\cdot}49^{CD}$	$0{\cdot}013^{C}$	$0{\cdot}23^{B}$	$9{\cdot}87^{C}$
Significance					
I	**	**	*	*	**
S	**	**	**	**	**
I × S	**	**	NS	NS	**
MSE	0·03	0·06	0·00002	0·05	0·83

CI = caecum inoculum; FI = faecal inoculum; CS = corn silage; AH = alfalfa hay; BG = barley grain; SM = soybean meal; BP = beet pulp; BCP = branched chain proportion, (isobutyric + isovaleric)/tVFA; I = inoculum effect; S = substrate effect; I × S = interaction between the effects. A, B, C, D and ** = $P < 0{\cdot}01$; a, b and * = $P < 0{\cdot}05$; NS = not significant; MSE = mean square error.

confirmed according to the findings of Bovera *et al*. (2006) in a study comparing the fermentation characteristics of three substrates obtained with caecal and faecal inocula of rabbits.

To shed light on the results reported in Table 4, it is worth pointing out that isobutyrate, isovaleric and valeric acids came, respectively, from the bacterial metabolism of the amino acids

Table 5. *Equations for estimation of caecal parameters from faeces (DF = 4)*

CI	FI	MSE	R^2	*P*-values
OMd=	7·43 + 0·91 OMd (5·31) (0·071)	4·51	0·9802	0·0011
OMCV=	4·41 + 0·84 OMCV (12·10) (0·04)	20·61	0·9919	0·0003
pH=	0·962 + 1·33 pH (1·14) (0·20)	0·003	0·9127	0·0112
R_{max}=	1·18 + 0·79 R_{max} (0·43) (0·06)	0·11	0·9820	0·0010
T_{max}=	5·02 + 0·61 T_{max} (2·61) (0·14)	2·61	0·8719	0·0203
NH_3=	3·47 + 2·19 NH_3 (0·70) (0·09)	6·61	0·9478	0·0051

CI = caecal inoculum; FI = faecal inoculum; OMd = organic matter digestibility; OMCV = gas cumulative volume related to organic matter incubated; R_{max} = maximum fermentation rate; T_{max} = time at which R_{max} is reached; MSE = mean square error.

valine, leucine and proline. Therefore, on the same fermented substrates, the caecal inoculum degraded the proteins more intensively, and also showed a higher synchronisation in nutrient fermentation: more intensive contemporaneous degradation of the carbohydrates (tVFA higher with CI) provides the bacteria with a greater quantity of carbon chains to use, in combination with the ammonia produced, for the synthesis of different amino acids.

Our observations, which certainly require further in-depth examination, suggest that the two inocula may behave according to the chemical characteristics of the substrates, especially structural carbohydrates. This aspect was also noted by Bovera *et al.* (2006) in a comparison of rabbit caecal content and rabbit faeces as source of inoculum for the IVGPT. Unfortunately, the lack of published data on the microbial population of ostrich faeces means that only hypotheses can be made. In fact, considering the differences in pattern of fermentation products (in particular propionate, butyrate and branched chain VFA), the microbial population of the two inocula are probably different. However, this difference did not appear to influence the rate and extent of OM digestion. Moreover, the micro-organisms in the faeces are in a state of 'suspended animation' (like the 'somnicell status' described by Roszak and Colwell, 1987). This may be due to many environmental factors, the most important being the lack of substrate available for the fermentations and the higher levels of O_2 to which some kinds of bacteria can be more sensitive. Corbett (1981) noted that the faeces of bovine, in respect of rumen liquor, had a lower content of micronutrients, essential for microbial activity.

Regarding the substrates, the mean values of degraded organic matter (OMd) found for alfalfa hay (55%) and barley (81%) were in agreement with the findings of Cilliers *et al.* (1997) who, using a balance ingesta–excreta method, found dry matter digestibility of 50 and 83%, respectively, for alfalfa and barley. The higher acetate production of barley may be surprising, given that beet pulp, alfalfa hay and corn silage have greater contents of structural carbohydrates. In the case of alfalfa and corn silage, the lower production of acetate can be justified by the lower digestibility of organic matter. For BP, probably, the fermentation of pectins induces propionate production in the ostrich caecum: Gebbink *et al.* (1999), in a study of the effects of beet pulp used as partial substitution for yellow corn and corn starch in pigs around weaning, observed that the production of propionate in the hindgut increased from 16·1 mmoles/l in the control group to 21·4 mmoles/l in the group fed on the diet containing 10% beet pulp.

Interestingly, barley and beet pulp, despite a similar protein concentration and organic matter digestibility, showed statistically significant differences in all the parameters reported in Table 4, in each case lower for BP. In the case of the VFA concentrations, the differences are due to the different amino acid composition. Indeed, valine and leucine are both higher for barley (val: 0·58 *vs* 0·44% a.f., respectively, for BG and BP; leu: 0·75 *vs* 0·56% a.f., respectively, for BG and BP, from Martillotti *et al.*, 1989).

Regarding the regression equations for estimating caecal fermentation characteristics from faeces, it is important to observe that for each inoculum, we compared the average data obtained from the 4 replications for each tested substrate. Hence, the number of observations was rather low. The estimation equations for OMd, OMCV, R_{max} and ammonia were statistically significant at $P < 0{\cdot}001$. For T_{max} and pH the estimation equations were significant at $P < 0{\cdot}05$. However, the equations have intercept values rather high, in particular, for OMCV, pH, R_{max} and T_{max}.

The use of faeces as an alternative source of inoculum to caecal content was viewed positively, although the need to undertake further research was underlined. The two inocula showed some differences *vis-à-vis* fermentation activity: the caecal inoculum fermented the substrates more quickly and, on the whole, more intensively, determining higher productions of total VFA (though not statistically significant), higher protein degradation and higher synchronism among the fermentations of carbohydrates and proteins. So, due to the differences in pattern of fermentation products, probably, caecal content and

faeces of ostriches had a different microbiology. However, the differences in fermentation pathways did not appear to affect the rate and extent of OM digestion.

The estimation equations are interesting but obtained from a small number of data. Undoubtedly, a larger number of analysed feeds could contribute to improving the equations. The estimation equations should be determined for 'classes' of feeds (concentrates, forages, by-products and diets).

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