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ORIGINAL ARTICLE

In vitro fermentation characteristics of different carbohydrate sources in two dog breeds (German shepherd and Neapolitan mastiff)

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Summary

Few studies have been published on the normal intestinal biota of canines unlike the wealth of information regarding livestock animal species. The *in vitro* gas production technique (IVGPT) including measurements of accumulating gas during fermentation and end-product determinations allows obtaining a complete picture of microbial activity kinetics. The aim of this study was to study the *in vitro* fermentation characteristics of different carbohydrate sources using *inocula* from two dog breeds (German Shepherd and Neapolitan mastiff). Faeces sampled from *rectum* of two GS and NM adult dogs, fed the same dry food, were used as *inocula*. The samples, diluted and filtered, were incubated at 39 °C under anaerobic condition with nine substrates different for carbohydrate composition (rice, corn, potato, spelt, pure cellulose, beet pulp, wheat bran, inulin and fructo-oligosaccharide). Gas production was recorded 17 times using a manual pressure transducer. After 48 h, the fermentation was stopped and fermenting liquor was analysed for pH and volatile fatty acids (VFA). Organic matter digestibility (OMD) was calculated as difference after burning the residuals. OMD, gas production and end-products were significantly correlated with chemical composition of substrates, in particular carbohydrate fractions (total dietary fibre and starch), confirming the effectiveness of the IVGPT in evaluating dog feeds. Concerning the comparison between breeds significant differences ($p < 0.01$) were found for OMD, gas production, fermentation kinetic parameters and end-products, suggesting a different pathway of fermentation and consequently, a different anaerobic population.

Introduction

A large population of micro-organisms, characterized by high density and wide diversity, inhabits the gut and forms a closely integrated ecological unit with the host. This complex mixed microbial culture could be considered as the most metabolically adaptable and rapidly renewable organ of the body (Simpson et al., 2002). The gut microbial community plays vital roles in physiological, nutritional and immunological function of the host (Mackie et al., 1997).

Even if the techniques developed by Hungate and refined by Bryant (1972) have allowed the publication of several information on the number, types and metabolic activities of bacteria that can be cultivated from the gut, only few papers have been published on the normal intestinal biota of canines (Clapper, 1970; Balish et al., 1977; Davis et al., 1977; Benno et al., 1992; Terada et al., 1992; Lewis

et al., 1994; Willard, 1996; Raskin et al., 1997), unlike the wealth of information regarding livestock species. Most of the studies on canine faecal bacteria have been carried out with Beagles and less is known about other canine breeds. On the other hand, the phylogenetically based classification scheme was recognized only recently and most of knowledge of gut bacterial populations has been described using indirect microbiological techniques such as selective plate counts, selective enrichment, pure culture isolation and most probable numbers of estimates. The most comprehensive study regards the characterization of canine faecal bacterial population in three dog breeds [German shepherd (GS), English setter and miniature Schnauzer], using both cultivation-based and molecular techniques (Simpson et al., 2002).

The *in vitro* gas production technique (IVGPT) proposed by Theodorou et al. (1994) measures the kinetics of fermentation and could be used to asses the activity of microbial populations (Williams et al., 2001). The IVGPT involves measurements of accumulating gas during fermentation, to assess microbial activity of the population acting as a whole. At the end of the fermentation period, samples are taken for the measurements of the end products [volatile fatty acids (VFA), other gas (NH_3)] and substrate utilization. The technique is carried out under strictly anaerobic conditions and is being used to estimate the activity of the microflora from many different sources, including rumen (Calabrò et al., 2004; Cutrignelli et al., 2007), different sections of gastrointestinal tract of pigs (Williams et al., 1997), rabbits (Bovera et al., 2008a,b), caecum of poultry (Williams et al., 1995; Bovera et al., 2007) and faeces of dog (Cutrignelli, 2006). By using different substrates, it becomes possible to look at shifts in microbial populations which are associated with the fermentation of a particular feedstuff. It could be questioned whether the use of faeces as *inoculum* for *in vitro* studies of *in vivo* intestinal events is truly representative for the fermentation earlier in the gastrointestinal tract (caecum or colon). Drasar (1988) described faeces as the spent culture *medium* of the large gut fermenter; it has been studied because it is readily available and provides a source material for the major groups of intestinal bacteria; it can also be sampled from the same host at different times. In detailed microbial count studies, Moore et al. (1978) concluded that the bacterial flora composition of faeces resembled that of large intestine and that freshly passed faeces collected under strictly anaerobic conditions could be considered as representative of large-intestinal flora. In terms of VFA and cumulative gas production, some differences were found between *inocula* from caecum and faeces (Williams et al., 1997), but it was concluded that faeces did nevertheless give a reasonable estimate of activity in the upper put of caecum-colon.

The aim of this study was to study the fermentation characteristics of different carbohydrate sources by IVGPT using faecal *inocula* from GS and Neapolitan mastiff (NM) dogs.

Material and methods

For the trial, two GS (3 years old) and two NM (3 years old) were utilized. The mean body weights of the animals were 32.5 kg and 59.8 kg for GS and NM respectively. The dogs raised in the same kennel were fed (140 kcal of ME/$kg^{0.75}$) a commercial dry food (crude protein 25.8% as fed; total dietary fibre 6.6% as fed). After 20 days of adaptation, faecal samples were collected *per rectum* and immediately transported, under anaerobic condition, to the laboratory.

Faecal samples were diluted (1:10) with NaCl solution, homogenized, filtered and incubated at 39 °C under anaerobic condition in 120 ml serum bottles (Bauer et al., 2001). Gas production of fermenting cultures was recorded 17 times (at 2–4 h intervals) using a manual pressure transducer. The fermentation was stopped after 48 h, and the fermenting liquor was analysed for pH (Alessandrini Instrument glass electrode; Jenway, Dunmow, UK; model 3030) and VFA. For VFA determination, the sample was centrifuged twice at 12 000 × *g* for 10 min at 4 °C and 1 ml of supernatant was taken and mixed with 1 ml of oxalic acid (0.06 mol). The VFA were measured by gas chromatography (ThermoQuest Italia SpA, Rodano, Milan, Italy; model. 8000top, fused silica capillary column 30 m × 0.25 mm × 0.25 μm film thickness) comparing samples peaks area of each VFA with the corresponding of an external standard composed by acetate, propionate, butyrate, isobutyrate, valerate and isovalerate (Calabrò et al., 2006).

Organic matter disappearance (OMD) was determined by filtering under vacuum on pre-weighed glass crucibles (Scott Duran, porosity #2) the fermentation residues, which was dried at 103 °C and burning the residual at 550 °C.

Gas volumes recorded during the fermentation were related to the quantity of incubated OM (organic matter cumulative volume, OMCV). The gas profiles were fitted to the model described by Groot et al. (1996) as follows:

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

where G: total gas produced (ml/g), A: asymptotic gas production (ml/g), B: time at which one-half of the asymptote has been reached (h), C: switching characteristic of the curve and t: time (h).

Maximum fermentation rate (R_{max}) and time at which it occurs (T_{max}) were also calculated according to the following formulas (Bauer et al., 2001):

$$R_{max} = (A \cdot C^B) \cdot B \cdot [T_{max}^{(-B-1)}]/[(1 + C^B)(T_{max}^{-B})^2]$$

$$T_{max} = C \cdot [(B-1)/(B+1)^{(1/B)}]$$

Nine substrates representative of carbohydrates with different fermentation characteristics were used: rice (R); corn (C); spelt (S); potato (P); sugar beet pulp (BP); wheat bran (WB); fructo-oligosaccharides (FOS); inulin (I) and pure cellulose (PC). Before the trial all substrates were analysed for the chemical composition (AOAC 2006); starch was determined using a polarimeter (Polax-L,model PX-L, ATAGO Co, LTD, Tokyo, Japan) (Martillotti et al., 1987).

The fermentation characteristics and the fitted parameters were subjected to analysis of variance (GLM procedure of SAS 2000) to detect the breed (MN and GS) and substrate (R, C, S, P, BP, WB, FOS, I and PC) effects; in the model the breed × substrate interaction was included. Pearson correlation coefficients between chemical composition and *in vitro* parameters and among the fermentation parameters were studied using the CORR procedure of SAS (2000).

Results

In Table 1, the chemical composition of the substrates is shown. Generally, chemical composition of the nine substrates agrees with the data reported in bibliography (INRA, Institut National de la Recherche Agronomique, 1987; Martillotti et al., 1989; Piccioni, 1989).

In Table 2, the fermentation characteristics are shown. As expected, IVGPT parameters were significantly influenced by substrates chemical composition: OMD was significantly ($p < 0.01$) higher for FOS and inulin (99.14 and 94.89% respectively), while cellulose gave the lowest value (2.50%). The substrates richer in starch (C, R, P, S) showed the highest values of OMCV. Concerning the mathematical model parameters rice, corn, spelt and potato showed significantly higher values of A, B, C and T_{max} than inulin and FOS, while the latest were characterized by significantly ($p < 0.01$) higher values of R_{max}.

Table 1 Chemical composition of the substrates (% as fed)

Substrate	Dry matter	Crude protein	Ether extract	TDF	Ash	Starch
R	87.53	6.49	0.66	6.42	0.53	73.43
C	88.39	7.76	1.03	8.04	1.41	70.15
P	87.81	8.45	0.21	13.2	4.78	61.16
S	88.20	13.7	1.66	12.2	1.50	59.24
PC	95.63	–	0.04	94.4	0.20	–
BP	90.48	7.51	0.55	77.8	3.43	1.170
WB	88.28	15.2	1.98	50.9	4.34	15.82
I	97.73	–	0.04	97.5	0.15	–
FOS	95.90	0.03	0.06	95.7	0.11	–

R, rice; C, corn; P, potato; S, spelt; PC, pure cellulose; BP, beet pulp; WB, wheat bran; I, inulin; FOS, fructo-oligosaccharides; TDF, total dietary fibre.

Concerning the comparison between breeds, the *inoculum* from NM gave significantly higher ($p < 0.01$) values of OMD (74.48 vs. 72.17%), B (16.15 vs. 12.56 h), C (2.45 vs. 2.15) and T_{max} (10.50 vs. 7.82 h) and lower of OMCV (138.9 vs. 157.6 ml/g) than GS.

Interesting correlation were found among the fermentation parameters and some chemical characteristics (Table 3). In particular, the correlation coefficients between TDF and several fermentation parameters gave high *r* values and were significant for OMCV, A ($p < 0.01$) and T_{max} ($p < 0.05$), while starch content was significantly ($p < 0.05$) correlated with OMCV, A and T_{max}. Protein and ether extract contents resulted significantly correlated with any fermentation parameter.

Values of pH measured at the end of the fermentation and VFA (mmol) concentration, and their correlation with chemical composition values were shown in Tables 4 and 5 respectively.

The pH determined after 48 h of incubation resulted particularly low for corn and inulin (4.52 and 4.72 respectively) and quite high for pure cellulose (7.06) and results significantly ($p < 0.01$) correlated with propionate (*r*: −0.676) and isovaleriate (*r*: 0.791) concentrations.

Volatile fatty acids concentrations were significantly correlated with chemical composition parameters, while carbohydrate fractions influenced ($p < 0.01$ and $p < 0.05$) acetate and propionate productions; crude protein and ether extract were related to isobutyrate, butyrate and valeriate production. Total VFA production resulted significantly ($p < 0.01$) higher for R, S, C and P (58.46, 58.00, 57.64 and 49.58 mmol respectively) than the other substrates, principally because of the higher concentrations of acetate and propionate. As expected, pure

Table 2 *In vitro* fermentation parameters of the substrates

	OMD (%)	OMCV (ml/g)	*A* (ml/g)	*B* (h)	T_{max} (h)	R_{max} (ml/h)
Substrate effect (mSD)						
R	$82.83^{B} \pm 6.09$	$175.2^{BCD} \pm 19.55$	$203.37^{A} \pm 28.16$	$21.44^{A} \pm 3.38$	$14.73^{A} \pm 4.77$	$7.08^{CD} \pm 0.84$
C	$86.46^{B} \pm 2.65$	$206.5^{A} \pm 30.04$	$208.14^{A} \pm 28.22$	$14.64^{C} \pm 5.41$	$9.11^{BC} \pm 4.38$	$10.78^{BC} \pm 3.8$
P	$88.29^{B} \pm 1.88$	$179.9^{ABC} \pm 8.70$	$209.87^{A} \pm 12.24$	$21.11^{AB} \pm 3.56$	$15.14^{A} \pm 3.33$	$7.53^{CD} \pm 0.94$
S	$88.15^{B} \pm 2.39$	$200.9^{AB} \pm 10.45$	$212.12^{A} \pm 10.87$	$17.09^{BC} \pm 3.16$	$13.79^{A} \pm 3.76$	$11.02^{BC} \pm 1.06$
PC	$2.50^{E} \pm 0.13$	$3.63^{E} \pm 0.17$	$3.53^{C} \pm 0.22$	$8.06^{D} \pm 2.70$	$3.91^{D} \pm 1.79$	$0.31^{E} \pm 0.16$
BP	$46.81^{D} \pm 3.25$	$129.1^{D} \pm 19.35$	$140.50^{B} \pm 12.09$	$23.72^{A} \pm 4.59$	$11.77^{AB} \pm 0.58$	$3.88^{DE} \pm 0.71$
WB	$63.56^{C} \pm 1.96$	$146.6^{CD} \pm 20.3$	$155.62^{B} \pm 12.49$	$6.31^{D} \pm 1.17$	$2.49^{D} \pm 1.12$	$16.06^{A} \pm 2.28$
I	$94.89^{A} \pm 2.76$	$158.3^{BCD} \pm 28.99$	$160.12^{B} \pm 30.16$	$8.06^{D} \pm 0.98$	$5.67^{CD} \pm 0.86$	$14.45^{AB} \pm 1.70$
FOS	$99.14^{A} \pm 0.78$	$134.0^{CD} \pm 33.32$	$140.71^{B} \pm 33.42$	$7.99^{D} \pm 1.41$	$5.35^{CD} \pm 0.84$	$13.6^{AB} \pm 2.84$
Breed effect						
NM	$74.48^{A} \pm 28.07$	$138.9^{B} \pm 60.04$	154.5 ± 66.0	$16.15^{A} \pm 8.00$	$10.50^{A} \pm 6.16$	8.64 ± 5.31
GS	$72.17^{B} \pm 29.20$	$157.6^{A} \pm 65.22$	163.3 ± 66.27	$12.56^{B} \pm 5.96$	$7.82^{B} \pm 4.25$	10.03 ± 5.69
Int.	0.3096	0.1751	0.0272	<.0001	0.0026	0.0380

Values are given as mean ± SD.

R, rice; C, corn; P, potato; S, spelt; PC, pure cellulose; BP, beet pulp; WB, wheat bran; I, inulin; FOS, fructo-oligosaccharides; OMD, organic matter digestibility; OMCV, organic matter cumulative volume; *A*, asymptotic gas production; *B*, time at which one-half of the asymptote has been reached; *C*, switching characteristic of the curve; R_{max}, maximum fermentation rate; T_{max}, time at which R_{max} occurs; GS, German shepherd; NM, Neapolitan mastiff; Int., interaction breed × substrate.

$^{A–E}p < 0.01$.

Table 3 Correlation among chemical characteristics and fermentation parameters

	OMD		OMCV		*A*		*B*		*C*		T_{max}		R_{max}	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p	*r*	p	*r*	p
TDF	0.643	NS	0.904	<0.01	0.935	<0.01	0.645	NS	0.571	NS	0.787	<0.05	0.261	NS
Starch	0.406	NS	0.687	<0.05	0.730	<0.05	0.518	NS	0.416	NS	0.682	<0.05	0.051	NS
CP	0.135	NS	0.520	NS	0.537	NS	0.305	NS	0.013	NS	0.315	NS	0.260	NS
EE	0.099	NS	0.428	NS	0.398	NS	−0.005	NS	−0.050	NS	0.019	NS	0.411	NS

TDF, total dietary fibre; CP, crude protein; EE, ether extract; OMD, organic matter digestibility; OMCV, organic matter cumulative volume; *A*, asymptotic gas production; *B*, time at which one-half of the asymptote has been reached *C*, switching characteristic of the curve; R_{max}, maximum fermentation rate; T_{max}, time at which R_{max} occurs; *r*, correlation coefficient; NS, not significant.

cellulose gave the lowest total VFA concentration (15.12 m); however, this substrate was characterized by higher valeric and isovaleric acids concentrations. Because of the lack of available nutrients, the microbial growth would be reduced and even microbial lysis could occur. It seems interesting to evidence that total VFA values for beet pulp and wheat bran resulted higher than inulin and FOS, as a result of the high fermentability of BP fibre and the relatively high starch content of WB (15.82% as fed). The low VFA production of FOS and I were probably caused by the nitrogen lack of these substrates.

At the end of the fermentation, the *inoculum* from NM produced more VFA than GS *inoculum* (47.51 vs. 38.78 mmol; $p < 0.01$) and the differences have to be ascribed exclusively to the propionate which in NM was approximately twice as in GS. On the other hand, the concentrations of isobutyric, isovaleric and valeric acids were significantly ($p < 0.01$) higher for GS than NM.

Figure 1 shows the gas production (ml/g) and the fermentation rate (ml/h) over time of the nine substrates in both breeds. GS inoculum was characterized by a faster initial rate and a higher gas production compared with NM. It seems interesting to underline that the *inocula* showed different trends for all substrates, particularly evident for those rich in starch (C, S, P and R), even though potato and rice showed similar curve shapes, but different from those of corn and spelt, probably because of the differences in starch fermentability. Regarding BP, PC and WB, the gas profiles of the two breeds were similar, while among feedstuffs the curve shapes were different. As expected, FOS and inulin gave similar

Table 4 Values of pH and fermentation end-products

	pH	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate mmol	Valerate
Substrate effect							
R	$5.35^{CD} \pm 0.20$	$26.17^{A} \pm 3.05$	$23.76^{A} \pm 3.09$	1.021 ± 0.64	$4.076^{AB} \pm 1.76$	$0.583A^{BC} \pm 0.03$	$0.992^{ABC} \pm 0.26$
C	$4.52^{E} \pm 0.17$	$23.26^{AB} \pm 3.84$	$23.58^{A} \pm 3.88$	0.967 ± 0.87	$4.898^{AB} \pm 1.16$	$0.408B^{C} \pm 0.185$	$0.746^{BC} \pm 0.14$
P	$5.55^{C} \pm 0.14$	$28.53^{A} \pm 3.52$	$21.77^{AB} \pm 2.94$	1.189 ± 0.83	$2.390^{AB} \pm 0.50$	$0.639A^{BC} \pm 0.41$	$0.901^{BC} \pm 0.36$
S	$5.42^{C} \pm 0.12$	$30.09^{A} \pm 3.41$	$26.27^{A} \pm 2.78$	1.422 ± 0.05	$4.076^{AB} \pm 0.717$	$0.966A^{BC} \pm 0.041$	$1.558^{AB} \pm 0.39$
PC	$7.06^{A} \pm 0.03$	$5.993^{B} \pm 1.39$	$1.898^{D} \pm 0.78$	0.997 ± 0.87	$0.802^{B} \pm 0.24$	$1.117^{A} \pm 0.24$	$1.037^{ABC} \pm 0.46$
BP	$6.25^{B} \pm 0.05$	$24.62^{A} \pm 3.79$	$11.37^{C} \pm 3.75$	1.073 ± 0.89	$1.847^{AB} \pm 0.42$	$0.769^{BC} \pm 0.08$	$1.497^{ABC} \pm 0.51$
WB	$6.29^{B} \pm 0.02$	$19.31^{AB} \pm 2.35$	$8.121^{CD} \pm 2.51$	1.497 ± 0.69	$7.418^{A} \pm 2.08$	$1.063^{AB} \pm 0.06$	$2.277^{A} \pm 0.44$
I	$4.72^{E} \pm 0.17$	$17.97^{AB} \pm 2.57$	$13.18B^{C} \pm 2.73$	1.218 ± 0.62	$0.670^{B} \pm 0.30$	$0.414^{BC} \pm 0.04$	$0.366B^{C} \pm 0.03$
FOS	$5.15^{D} \pm 0.27$	$17.39^{AB} \pm 3.11$	$11.62^{C} \pm 1.99$	0.642 ± 0.27	$2.069^{AB} \pm 0.13$	$0.300^{C} \pm 0.13$	$0.197^{C} \pm 0.06$
Breed effect							
NM	5.60 ± 0.83	22.29 ± 8.62	$20.23^{A} \pm 12.67$	$0.595^{B} \pm 0.289$	3.402 ± 2.84	$0.4688^{B} \pm 0.31$	$0.522^{B} \pm 0.54$
GS	5.57 ± 0.74	20.67 ± 13.49	$11.23^{B} \pm 6.71$	$1.633^{A} \pm 1.04$	2.717 ± 0.91	$0.923^{A} \pm 0.52$	$1.605^{A} \pm 1.18$
Int.	<0.0001	0.3458	<0.0001	0.4710	0.5757	0.9281	0.2347

Values are expressed as mean ± SD.

R, rice; C, corn; P, potato; S, spelt; PC, pure cellulose; BP, beet pulp; WB, wheat bran; I, inulin; FOS, fructo-oligosaccharides; GS, German shepherd; NM, Neapolitan mastiff; Int., interaction breed × substrate.

$^{A–E}p < 0.01$.

Table 5 Correlation among chemical characteristics, pH and end-products

	pH		Acetate		Propionate		Isobutyrate		Butyrate		Isovalerate		Valerate	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p	*r*	p	*r*	p
TDF	−0.529	NS	0.952	<0.01	0.942	<0.01	0.338	NS	0.489	NS	−0.251	NS	0.192	NS
Starch	−0.400	NS	0.695	<0.05	0.865	<0.01	0.228	NS	0.537	NS	−0.165	NS	0.139	NS
CP	0.083	NS	0.632	NS	0.417	NS	0.736	<0.01	0.819	<0.01	0.404	NS	0.827	<0.01
EE	0.041	NS	0.401	NS	0.282	NS	0.686	<0.01	0.895	<0.01	0.509	NS	0.800	<0.01

TDF, total dietary fibre; CP, crude protein; EE, ether extract; *r*, correlation coefficient; NS, not significant.

trend in terms of gas production and fermentation rate.

In Table 6, the correlations between fermentation parameters and end-products were reported. OMD was significantly ($p < 0.01$) correlated with OMCV and the ratio among acetate plus butyrate (the VFA more involved in gas production), and propionate (the VFA which give less gas production). OMCV was significantly ($p < 0.01$) related with all considered parameters.

Discussion and conclusion

The gas production and the OM digestibility registered within 48 h with GS *inoculum* for pure cellulose, FOS, inulin and wheat bran were in accordance with that of a previous study (Cutrignelli, 2006) carried out using faecal *inoculum* from the same breed. The high correlation among carbohydrate composition (TDF and starch) and *in vitro* parameters confirm the suitability of IVGPT to study the fermentation characteristics of feed for dogs.

The *inocula* showed low cellulolytic activity, in both case PC was digested only in minimal portion, according to Sunvold et al. (1995), while the percentages of OMD for FOS, I and for substrates rich in starch (P, S, C and R) were high, demonstrating high microbial activity on non-structural carbohydrates.

However, the differences registered between breeds in fermentation parameters, end-products and fermentation kinetics suggest different pathways of carbohydrate fermentation, in particular for substrates rich in starch and confirm the observation of Simpson et al. (2002), who found significant differences among breeds on selected aerobic and anaerobic bacterial counts.

Faeces from NM degraded the OM more intensively, but gas produced along the fermentations (OMCV, A) was lower than from GS faeces. However, the higher OM degradation was not imputable

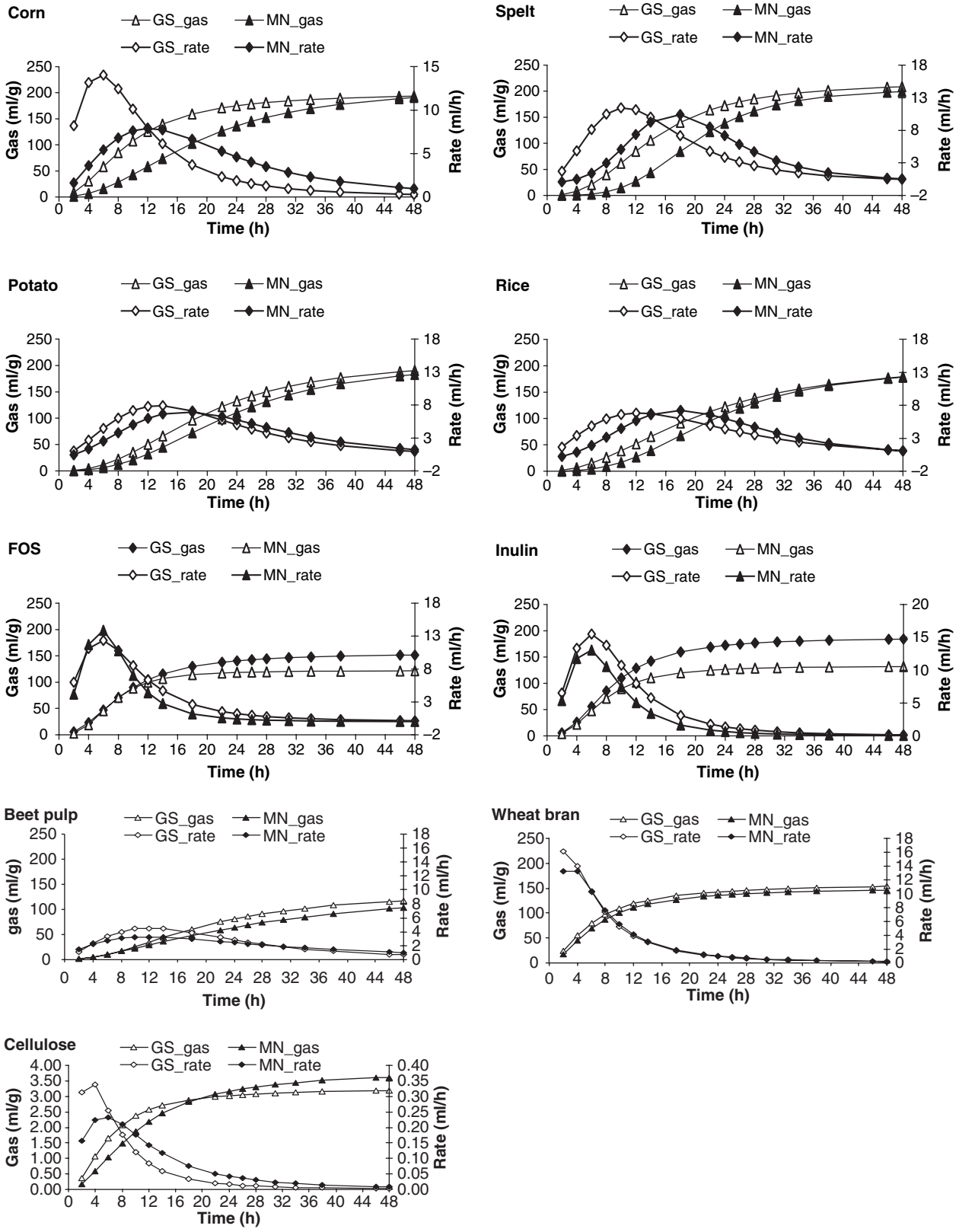

Fig. 1 *In vitro* gas production and fermentation rate over time of the substrates in German shepherd (GS) and Neapolitan mastiff (NM) dogs.

Table 6 Correlation among the *in vitro* fermentation parameters

	OMCV		VFA		(Ace + But)/Prop	
	r	p	*r*	p	*r*	p
OMD	0.8777	<0.01	0.6427	NS	−0.844	<0.01
OMCV	–	–	0.9044	<0.01	0.810	<0.01

OMD, organic matter digestibility; OMCV, organic matter cumulative volume; VFA, total volatile fatty acid; (Ace + But)/Prop, (acetate + butyrate)/propionate; *r*, correlation coefficient; NS, not significant.

to protein degradation, but to the different utilization of non- structural carbohydrates. It is worth pointing out that isobutyric, isovaleric and valeric acids came from the bacterial metabolism of valine, leucine and proline respectively. Therefore, on the same fermented substrates, faeces from GS degraded the proteins more intensively than NM. The non-significant interactions between the effects suggest that the *inocula* have the same behaviour, irrespective of the differences among substrates. Indeed faecal *inoculum* from NM seems more able to utilize some substrates producing high proportion of propionate and consequently low gas. It is known that the production of propionate is tied to a lower production of gas (CO_2). Actually, acetate production is correlated with one molecule of CO_2 and one of H_2, while that one of propionate is only correlated with 1 *molecule* of H_2O. In this case, the interaction was statistically significant due to the particular behaviour of cellulose which showed an inverse trend compared with the other substrates, because it was the only one purified. As a consequence, the two breeds seem very different in terms of fermentation capability, probably due to a different microbial population or different microbial activity.

These preliminary results could be considered to be the first step to study the gastrointestinal microbiota activity in dogs using the gas production technique.

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