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METHANOGENESIS IN RABBIT CAECUM AS AFFECTED BY THE FERMENTATION PATTERN: *IN VITRO* AND *IN VIVO* MEASUREMENTS

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Abstract: Methane formation and caecal fermentation patterns were studied *in vivo* and *in vitro* in 16 New Zealand White rabbits (70-80 d and 2.27±0.064 kg) allocated to 4 diets formulated to have similar neutral detergent fibre (33.8±0.53%) and crude protein (17.7±0.33%) content, with 2 different sources of fibre (alfalfa hay, AH; or sugar beet pulp, SP) and starch (wheat or maize). Animals received the diet for 16 to 20 d before methane production was measured *in vivo* in a respiratory chamber. Animals were subsequently slaughtered at approximately 9:00 a.m. and caecal contents were sampled and used as inoculum for *in vitro* incubations to determine gas and methane production. Volatile fatty acid (VFA) and purine base (PB) concentrations were determined from both caecal content and incubation medium after 6 h. Total VFA concentration in caecal content decreased (P<0.05) in rabbits fed AH-maize diet compared with rabbits fed AH-wheat and SP-maize diets (37.7 *vs*. 59.6 mM), with those fed SP-wheat showing an intermediate value (53.0 mM). Fermentation pattern was affected when maize was the source of starch compared to wheat, with lower acetate (0.72 *vs*. 0.79; P<0.01) and higher butyrate (0.19 *vs*. 0.14; P<0.001) molar proportions. Fermentation *in vivo vs*. *in vitro* showed some differences (molar proportions of acetate, 0.76 *vs*. 0.73, P<0.001, and propionate, 0.069 *vs*. 0.091, P<0.001, *in vivo* and *in vitro*, respectively), probably due to differences in pH (6.0 *vs*. 6.7 *in vivo* and *in vitro*; P<0.001). Only 2 out of 16 rabbits produced a substantial volume of methane *in vivo* (on average, 12.6 mL or 0.56 mmol per kg of metabolic weight and day), showing a high inter-individual variability that hindered comparison of treatment differences. In contrast, methane was detected *in vitro* in all cases and volumes were more homogenous, a higher formation (P<0.05) being observed with maize compared to wheat. A similar effect was shown in total gas production. The low methane production and H_2 recovery suggest the importance of H_2 disposal mechanisms other than methanogenesis, such as reductive acetogenesis. PB concentration in caecal content and the incubation medium, as an index of microbial concentration, was highest when SP was added with maize (P<0.05).

Key Words: rabbit, caecal fermentation, methanogenesis, reductive acetogenesis.

INTRODUCTION

In rabbits, the caecum represents the main fermentation site, where microorganisms convert nutrients leaving the small intestine to volatile fatty acids (VFA), gases (CH_4, CO_2, H_2), ammonia and compounds incorporated into microbial cells. Hydrogen is formed during fermentation, but

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a high partial pressure of H_2 in some anaerobic ecosystems reduces the fermentation efficiency (Wolin *et al*., 1997). There are a number of alternative pathways for H_2 disposal in the digestive tract sites, mainly methanogenesis and reductive acetogenesis. Literature data indicate a competition for metabolic H_2 uptake between methanogenic Archaea and acetogenic bacteria in the animal digestive tract (Bernalier *et al*., 1993; De Graeve *et al*., 1994). Whereas in adult ruminants methanogenesis is the main H_2 sink, in monogastric animals both reductive acetogenesis and methanogenesis may occur together with other possible pathways for disposal of H_2 (Jensen, 1996). Caecal fermentation has been extensively studied in rabbit, but methanogenesis has been reported only in a few *in vitro* studies, and recently Franz *et al*. (2010) reported *in vivo* measurements of methane production in adult rabbits of 0.20 L/d. Those *in vitro* experiments suggested that methane production is almost absent from fermentation before weaning (Piattoni *et al*., 1996; Marounek *et al*., 1999), and that reductive acetogenesis is a major characteristic of caecal fermentation in kits, being partially replaced from 6 wk of age by methanogenesis, coinciding with the increasing intake of solid food. However, reductive acetogenesis still appeared to be important in other *in vitro* studies using some older individuals (Marounek *et al*., 1999). Factors involved in the partitioning of H_2 between reductive acetogenesis and methanogenesis in the rabbit caecum are not well understood, and might be affected by diet as well as caecal fermentation. The aim of this work was therefore to investigate in *in vivo* and *in vitro* experiments the effect of different sources of fibre and starch on caecal fermentation pattern and methane production in rabbits.

MATERIALS AND METHODS

Animals and experimental design

Sixteen New Zealand White rabbits (70-80 d and 2.27±0.064 kg) were randomly allocated to 4 different diets, formulated with a similar concentration of neutral detergent fibre (NDF) but different in fermentable carbohydrates, which were based on 2 fibre sources (alfalfa hay, AH; and sugar beet pulp, SP) and 2 sources of starch (wheat and maize). Ingredients and chemical composition of diets are shown in Table 1. Feed was provided daily at 8:00 a.m. at a restricted level (100 g/d) in order to minimise differences in caecal fermentation due to different ingestion. Animals were maintained under a constant light cycle of 12 h of light (8:00 a.m. to 8:00 p.m.) and 12 h dark , and had free access to fresh drinking water. Each animal received the experimental diet at a restricted level for 16 d before being individually confined for 3 consecutive periods of 7 to 8 h in a hermetically sealed respiratory chamber. Methane production per min was estimated from the change in its concentration within the fixed volume of the chamber as described by Lachica *et al*. (1995) and then the average formation of the 3 periods was calculated and expressed per day. Next, all animals were slaughtered at 9:00 a.m. and the caecum excised and dissected. The pH of caecal contents was measured and samples were taken and stored frozen for determination of VFA (1 g in 1 mL of 0.15 mol H_3PO_4/L with 2 mg/mL of 4-methylvaleric acid as internal standard) and purine bases (PB; 4 g) concentration. The remaining caecal contents were used as inoculum for *in vitro* incubations.

Ninety g of caecal content from each rabbit (16 different inoculums from 4 rabbits/diet) were diluted in 900 mL of incubation medium (Theodorou *et al*., 1994) under anaerobic conditions, and this solution was used as inoculum for the *in vitro* incubations. The *in vitro* trial was conducted in nine 120 mL glass bottles, fitted with a rubber stopper and aluminium crimp seal. For each inoculum, 3 bottles were used for gas production measurements and another 3 for gas

and medium sampling. Sterilised caecal contents (800 mg added to each bottle) were obtained for use as substrate by slaughtering four 7-8 month-old female rabbits that had each received one of the four experimental diets for one week. The last three bottles, without substrate, were also included as blanks. Each bottle was filled with 90 mL of inoculum (inital pH 6.7) and incubated at 39ºC in anaerobic conditions. The average of the three bottles per rabbit was considered as the experimental unit.

The microbial fermentation pattern was studied by measuring the gas produced during the *in vitro* incubation of substrate (Theodorou *et al*., 1994) and VFA and methane production were determined. The gas pressure produced in three bottles per treatment was recorded on a HD8804 manometer with a TP804 pressure gauge (DELTA OHM, Italy) after 2, 4 and 6 h of incubation. Readings were converted into volume (mL) by using the following pre-established linear regression between pressure recorded (mbar) in the same type of bottles and known headspace volumes (Marinas *et al*., 2003):

$$\text{volume}=(\text{pressure}-0.981)/30.375 \quad (r = 0.996;\ n = 64)$$

After 6 h of incubation, total gas production was also measured in the other three bottles of each treatment and a gas sample (about 15 mL) was removed and stored in a Haemoguard Vacutainer

Table 1: Ingredients and chemical composition of diets (on a fresh matter basis).

Fibre source	Alfalfa hay		Sugar beet pulp	
Starch source	Maize	Wheat	Maize	Wheat
Ingredients (%)				
Sugar beet pulp	19.6	18.5	47. 8	48.5
Alfalfa hay	50.6	51.6	15.9	16.2
Maize	18.4	0	14.9	0
Wheat	0	19.6	0	16.5
Soybean meal	10.7	9.7	16.1	14.6
NaOH-treated straw	0.5	0	5.1	4.1
Sunflower oil	0	0.4	0	0
Mineral-vitamin mixture[1]	0.2	0.2	0.2	0.2
Chemical composition (%)				
Dry matter	91	91.2	90.6	91
Organic matter	89.9	89.9	92	92.2
Crude protein	18.1	18.4	17	17.4
Soluble fibre[2]	12.7	12.6	21.9	22.4
Starch[3]	13	13	10	10.3
Neutral detergent fibre	33.8	32.4	35	34.1
Acid detergent fibre	17.6	18.1	18.2	18.1
Lignin	4.6	4.1	2. 8	2.6

[1] Composition of mineral-vitamin mix: 200 mg/kg Co ($CoSO_4$ 7 H_2O), 3 g/kg Cu ($CuSO_4$ 5 H_2O), 20 g/kg Fe ($FeSO_4$ 5 H_2O), 8 g/kg Mn (MnO_2), 30 g/kg Zn (ZnO), 30 mg/kg Se (Na_2SeO_3), 500 mg/kg I (KI), 4500000 IU/kg vit A, 550000 IU/kg vit D_3, 1100 mg/kg vit E, 250 mg/kg vit B_1, 1500 mg/kg vit B_2, 100 mg/kg vit B_6, 6000 mg/kg vit B_{12}, 500 mg/kg vit K, 5000 mg/kg D-pantothenate, 12.5 g/kg niacin, 100 g/kg choline chloride. [2] Values estimated from Bach Knudsen (1997). [3] Values estimated from FEDNA (2003).

(Terumo Europe N.V., Leuven, Belgium) before analysis for CH_4 concentration. Bottles were then uncapped, the pH was measured immediately with a pH meter, and the incubation medium of these bottles was sampled to determine the VFA (4 mL, acidified with 1 mL of 0.5 mol/L H_3PO_4) and PB (6 mL) concentration.

Chemical analyses

Chemical analyses of feeds were carried out following the AOAC (2005) procedures on dry matter (DM; 934.01), organic matter (OM; 942.05), crude protein (CP; 976.05) and ether extract (EE; 2003.05). NDF and acid detergent fibre (ADF) and lignin were determined in a Fibertec 1020 System (Foss Tecator, Höganas, Sweden) according to Van Soest *et al*. (1991). The NDF and ADF were expressed exclusive of residual ash and an α–amylase was used in the NDF analysis. The neutral detergent solution did not contain sodium sulphite. VFA concentration was analysed by gas liquid chromatography following the procedure described by Jouany (1982). Methane concentration was analysed by injecting 0.5 mL of gas into a gas chromatograph (Shimadzu GC 14B; Shimadzu Europa GmbH, Duisburg, Germany) equipped with flame ionisation detector and a column packed with Carboxen 1000 (Supelco, Madrid, Spain) as described by Martínez *et al*. (2010). Purine base (PB, adenine and guanine) concentration was analysed by high performance liquid cromatography after hydrolysis of the samples with 2 mL of 2 mol/L $HClO_4$ at 100 ºC for 1 h, with previous addition of allopurinol as internal standard and neutralising immediately with 4 mol/L KOH (Martín-Orúe *et al*. 1995).

Calculations and statistical analysis

Net production of VFA *in vitro* was calculated by subtracting the amount initially present in the inocula from that determined in the content cultures after 6 h of incubation. Methane production *in vitro* was calculated by multiplying gas produced after 6 h of incubation by the concentration of methane in the sample analysed.

Hydrogen recoveries ($2H_{rec}$) were calculated according to Demeyer (1991) as:

$$2H_{rec}=100\times(2P+2B+4M)/(2A+P+4B)$$

where A, P, B and M represent net production (μmol) values of acetate, propionate, butyrate and methane, respectively.

Data were analysed in a completely randomised design, following a 2×2 factorial structure. The fibre and starch sources were included as fixed effects and the animal as a random effect that was nested within the interaction of the fixed effects. In order to compare *in vivo* and *in vitro* results from the same animal, the approach was included in the model as a fixed effect (*in vivo vs*. *in vitro*) and the animal as a random effect. Whether samples have the same distribution was examined by the Kolmogorov-Smirnov test. All statistical analyses were performed using the MIXED procedure of the SAS software package, version 9.1 (SAS Institute, Cary, NC, USA). Treatment differences were contrasted by the least significant difference test, and differences among means with $P<0.05$ and $0.05<P<0.10$ were accepted as representing statistically significant differences and differences close to significance, respectively.

RESULTS AND DISCUSSION

Caecal concentration of the total VFA and the molar proportions of the main VFA *in vivo* and *in vitro* are shown in Table 2. Total concentration of VFA in caecal contents was affected by the source of starch only when AH was the main source of fibre, being lower with maize (interaction

Fibre×Starch; P=0.030). Previous results (Belenguer *et al*., 2002) with similar diets showed no differences between fibre (alfalfa hay *vs*. sugar beet pulp) or grain (maize *vs*. barley) sources. Conversely, total VFA concentrations *in vitro* were not significantly affected by the experimental treatments and showed an average value of 16.4±0.88 mM. The Kolmogorov-Smirnov test indicates that responses in VFA concentration differed between *in vitro* and *in vivo* approaches (P<0.05). These differences might indicate either changes in microbial fermentative activity due to pH differences (6.0 *vs*. 6.7 *in vivo* and *in vitro*, respectively; P<0.001) or a possible influence of factors that cannot be studied *in vitro*, such as transit time. In this sense, the greater dietary lignin of AH compared to SP could be associated with a faster rate of passage (Gidenne *et al*., 2001) and AH is more lignified than SP, and this might have prevented the caecal fermentation of some of the less digestible components of maize.

The VFA pattern from fermentation was affected by diet, either *in vivo* or *in vitro*, and rabbits given wheat presented higher acetate (P<0.01) and lower butyrate (P<0.001) molar proportions than those fed maize. In addition to proportion and composition differences from the fibrous fractions of both cereal grains studied, maize includes a sizeable resistant starch fraction (Blas and Gidenne, 1998), which might lead to a higher amount of starch from maize reaching the caecum, even though the high starch ileal digestibility in rabbits (Gidenne and Perez, 1993) prevents a large input to the caecum in any case. This would explain a more butyrogenic fermentation (Bird *et al*., 2007). In this regard, previous studies did not detect major differences among starch sources in the VFA profile (Belenguer *et al*., 2002). Furthermore, *in vitro* molar proportions of propionate were higher in rabbits fed AH diets compared to those receiving SBP (P<0.05). *In vivo* molar acetate and propionate proportions were respectively higher (0.76 *vs*. 0.73; P<0.001)

Table 2: Effect of fibre and starch sources on concentration of total volatile fatty acids (VFA), molar proportions of the main VFA and pH in caecal contents, and in 6-h *in vitro* cultures using the same caecal contents as inocula.

Fibre source	Alfalfa hay		Sugar beet pulp			*P*-value		
Starch source	Maize	Wheat	Maize	Wheat	SEM	Fibre (F)	Starch (S)	F×S
Caecal contents:								
Total VFA (mM)	37.7[a]	58.0[b]	61.2[b]	53.0[ab]	5.8	NS	NS	0.031
Molar proportion:								
Acetate	0.722	0.791	0.721	0.785	0.017	NS	0.002	NS
Propionate	0.074	0.072	0.069	0.061	0.008	NS	NS	NS
Butyrate	0.19	0.13	0.199	0.148	0.011	NS	<0.001	NS
pH	6.17	5.94	6.08	5.85	0.21	NS	NS	NS
In vitro cultures:								
Total VFA (mM)	14.9	16.4	16.7	17.6	1.9	NS	NS	NS
Molar proportion:								
Acetate	0.700	0.742	0.702	0.759	0.012	NS	0.001	NS
Propionate	0.097	0.101	0.085	0.081	0.007	0.037	NS	NS
Butyrate	0.181	0.136	0.197	0.147	0.009	NS	<0.001	NS
pH	6.75	6.73	6.72	6.66	0.02	0.028	0.084	NS

SEM: standard error of means (n=4). NS: P>0.10. Mean values in the same row not sharing the same superscript differ at P<0.05. Superscripts are shown only when a significant (P<0.05) Fibre×Starch interaction was detected.

and lower (0.069 *vs*. 0.091; $P<0.001$) than *in vitro*, probably due to pH differences, as commented previously. It should be pointed out that although pH was affected by the experimental treatment *in vitro*, this was probably due to the low variability observed in this parameter (CV=0.73 %), and these effects were not significant *in vivo*.

Both *in vivo* and *in vitro* approaches have drawbacks that may affect the soundness of results. Slaughter trials assume that all animals are at the same physiological stage, which largely depends on intake and time after feeding. In contrast, pH in batch cultures is kept within 6.5 and 7.0, because of the limited range of action of the bicarbonate buffer used (Goering and Van Soest, 1970), although it is not advisable to maintain pH below 6.5 (Kohn and Dunlap, 1988).

Methane formation *in vivo* (Table 3) was low throughout treatments, except for 2 rabbits given a maize diet that produced substantial volumes per day (on average, 12.6 mL or 0.56 mmol per kg of metabolic weight ($BW^{0.75}$) and day. In consequence, the individual variability in methane production *in vivo* was very high, with a coefficient of variation (CV) of 245 %, which helps explain the lack of significant differences. Franz *et al*. (2010) observed a much higher methane production (143 mL/$BW^{0.75}$/d), differences that these authors associate with the use of adult animals given grass hay (64% NDF) as the only feed. However, it is worth noting the important variability (CV=54 %) that was observed. On the other hand, *in vitro* methane represented on average 0.81% of the total gas produced, and its production was less variable than *in vivo* (CV=16%). No treatment differences ($P>0.05$) were observed in the methane produced *in vitro*, but the inocula from rabbits given maize diets tended ($P=0.09$) to produce more methane than those from rabbits given the wheat diets. When expressed on substrate weight basis, again fibre sources did not affect methane production and the effect of starch became significant, which

Table 3: Effect of fibre and starch sources (alfalfa hay *vs*. sugar beet pulp and maize *vs*. wheat) on *in vivo* and *in vitro* methane production, *in vitro* H_2 recovery, and *in vitro* gas production (mL/g substrate) over time in rabbits.

Fibre source	Alfalfa hay		Sugar beet pulp			*P*-value		
Starch source	Maize	Wheat	Maize	Wheat	SEM	Fibre (F)	Starch (S)	F×S
Methane *in vivo* (per d):								
mL/$BW^{0.75}$	5.20	0.28	1.63	0.77	2.45	NS	NS	NS
mmol/$BW^{0.75}$	0.232	0.013	0.072	0.034	0.110	NS	NS	NS
Methane *in vitro* (in 6 h):								
µl/mL gas	8.81	7.55	8.58	7.56	0.62	NS	0.094	NS
µmol/mL gas	0.394	0.338	0.383	0.338	0.028	NS	0.094	NS
µl/g substrate	211.7	159.1	232.3	164.2	21.2	NS	0.009	NS
µmol/g substrate	9.46	7.11	10.38	7.34	0.95	NS	0.009	NS
H_2 recovery (%)	26.24	23.75	27.12	22.17	1.30	NS	0.016	NS
Gas prod. *in vitro*:								
2 h	8.48	7.48	9.25	7.19	0.82	NS	0.04	NS
4 h	16.83	15.56	18.56	15.01	1.28	NS	0.071	NS
6 h	23.87	21.07	25.97	21.72	1.51	NS	0.025	NS

SEM: standard error of mean (n = 4). NS: $P>0.10$. Mean values in the same row not sharing the same superscript differ at $P<0.05$.

might be due to potential differences in substrate utilisation rates (e.g., wheat starch is more rapidly degradable than maize starch).

The total volume of gas produced *in vitro* from caecal contents (Table 3) increased with time up to 6 h, and was not affected ($P>0.10$) by the fibre source in the diet at any sampling time. In contrast, maize induced a higher gas production than wheat ($P<0.05$ at 2 and 6 h, and $P=0.07$ at 4 h).

Methanogenic microorganisms have been described in the caecum of adult rabbits and are diverse. According to Bennegadi *et al*. (2003), the Archaeal community comprises up to 22 % of total microbial RNA in 70 d-old rabbits and is largely dependent on the dietary fibre level. Michelland *et al*. (2010) reported a richness index around 10, estimated by CE-SSCP in 84 d old rabbits. Nevertheless, in contrast to the rumen, where methane production is one of the main pathways to dispose of reducing equivalents generated during fermentation, methanogenic organisms are less abundant in the large intestine (Morvan *et al*., 1996). Competition among 3 main H_2-consuming organisms, methanogenic Archaea, acetogenic bacteria and sulphate reducing bacteria, has been described in the large intestine. The latter organisms have an initial competitive advantage because of their higher substrate affinity for H_2 than methanogenic Archaea (Gibson *et al*., 1990), but their growth depends on sulphate availability. An alternative route for H_2 disposal is reductive acetogenesis, although the relative substrate affinities of methanogens for H_2 ought to favour methanogenesis in a competitive environment (Macfarlane and Gibson, 1997). Therefore, methanogenesis should usually dominate H_2-dependent acetate production in anaerobic ecosystems, although the greater presence of acetogenesis in rabbits might lie in the higher acid sensitivity of sulphate reducing bacteria and methanogens (Gibson *et al*., 1990). Methane production is strongly affected by pH, and Russell (1998) showed that methane production *in vitro* decreased dramatically at pH below 6.3. This may also explain the greater methanogenic activity *in vitro vs*. *in vivo* due to the higher pH *in vitro* (6.7 *vs*. 6.0). Moreover, acetogens can grow better in a low-substrate environment due to their ability to grow on substrates other than H_2/CO_2, such as monosaccharides (Drake, 1994), and are more resistant to bile salts (Jezierny *et al*., 2007). These capacities would allow them to be more competitive than methanogens in a digestive tract with a lower pH and a fast passage rate (Morvan *et al*., 1996), as observed in rodents by Doré *et al*. (1995), and might explain the lower presence of methanogens in the caecum and colon of rabbits than in fermentation compartments of other host species.

The *in vitro* approach allows for further H_2 disposal investigation and indeed the low hydrogen recovery values usually reported for *in vitro* fermentations of caecal contents suggest a greater importance of other pathways of H_2 disposal than methanogenesis, such as reductive acetogenesis. Our values for H_2 recovery ($2H_{rec}$) ranged from 22.2 to 27.1 % (Table 3) and are lower than those reported by Piattoni *et al*. (1996) in 56 d old rabbits (50 %), but similar to those observed by Marounek *et al*. (1999) (26.0 to 29.6 % in rabbits 11 wk old). Hydrogen recovery was higher ($P<0.01$) when maize was used as source of starch, probably due to a higher methane production. Previous *in vitro* studies (Piattoni *et al*., 1996) have suggested that reductive acetogenesis may be the main H_2 sink in the caecum of kits, but it can be replaced gradually and partially by methanogenesis after weaning according to intake of solid food. Methanogenesis seems to be almost absent in animals before weaning, although it increases afterwards (Marounek *et al*., 1999). In any case, it must be considered that only a small fraction of the methanogens making up a natural community can generally be cultured because of their fastidious culture requirements, extremely low growth rates and obligate anaerobiosis (Raskin *et al*., 1994) and this may bias

Table 4: Effect of fibre and starch sources on purine base concentration (μmol/g dry matter) as an index of microbial concentration in the caecal contents of rabbits and in 6 h *in vitro* cultures using the same caecal contents as inocula.

Fibre source	Alfalfa hay		Sugar beet pulp			*P*-value		
Starch source	Maize	Wheat	Maize	Wheat	SEM	Fibre (F)	Starch (S)	F×S
Caecal contents	250.5^{b}	322.0^{b}	464.2^{a}	300.3^{b}	47.0	0.054	NS	0.023
In vitro cultures	19.4^{b}	25.0^{b}	36.4^{a}	27.4^{b}	2.6	0.003	NS	0.016

SEM: standard error of mean (n = 4). NS: $P>0.10$. Mean values in the same row not sharing the same superscript differ at $P<0.05$.

comparison of results from *in vivo* and *in vitro* approaches. In any case, although methanogenic archaea exist in the rabbit caecum, only some rabbits exhibited a remarkable CH_4 production, which might indicate the existence of a potential genetic effect, as suggested by Piattoni *et al*. (1995), although this is not clear and should be further studied.

Concentration of PB was determined in both caecal contents and *in vitro* cultures after 6 h of *in vitro* incubation as an index of microbial concentration (Table 4). In both cases, SP induced a higher ($P<0.10$ and 0.01 *in vivo* and *in vitro*, respectively) concentration compared to AH, but these differences were only significant when maize was included as the source of starch (interaction Fibre×Starch; $P<0.05$). As mentioned above, a greater input of resistant starch from maize than from wheat may reach the caecum. This, together with the estimated higher ingestion of soluble fibre from sugar beet pulp may stimulate microbial activity, as has previously been observed (Belenguer *et al*., unpublished). However, other previous studies (Belenguer *et al*., 2002) did not show any effect of fibre or starch sources on microbial PB concentration in the rabbit caecum.

CONCLUSIONS

In conclusion, this study indicates that, unlike fermentation in other species, methanogenesis may not be the major H_2 sink in rabbit caecal environment. However, methane formation could become remarkable *in vitro* with a pH closer to neutrality, which seems to be favourable, supporting the assertion that methanogenic Archaea exist in caecal contents. Methane production cannot be associated with diet characteristics *in vivo* because of the high individual variability, although the source of starch modulated volatile fatty acid profile and thus affected caecal methanogenesis *in vitro*.

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