Spanish Journal of Agricultural Research
15(2), e0602, 8 pages (2017)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2017152-10753
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

In vitro fermentation pattern and acidification potential of different sources of carbohydrates for ruminants given high concentrate diets

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Abstract

The *in vitro* fermentation pattern of five sources of carbohydrates of differing nature (maize grain, MZ; sucrose, SU; wheat bran, WB; sugarbeet pulp, BP; and citrus pulp, CT) under conditions of high concentrate diets for ruminants was studied. A first 8 h incubation trial was performed under optimal pH using inoculum from ewes given a fibrous diet, to compare fermentative characteristics of substrates. As planned, incubation pH ranged within 6.3 to 6.6. The gas produced from CT was higher than MZ, SU and BP from 4 and 6 h onwards, and at 8 h, respectively ($p<0.05$). There were no differences ($p>0.05$) on total volatile fatty acid (VFA) concentration, nor on acetate or propionate proportions, but butyrate was lowest ($p<0.05$) with CT and BP. The second incubation trial was performed in a poorly-buffered medium, with inoculum from ewes given a concentrate diet. All substrates showed a gradual drop of pH, being lowest with SU after 4 h ($p<0.05$). Throughout the incubation, gas production was highest with CT and lowest with MZ and BP ($p<0.05$). Total 8 h VFA concentration was higher with CT than BP, SU and MZ ($p<0.05$). Acetate proportion was higher, and that of propionate lower, with BP than WB ($p<0.05$), butyrate proportion being higher with MZ and WB than with BP and CT ($p<0.05$). Lactic acid concentration was higher ($p<0.05$) with SU than WB and BP. Fermentation characteristics and acidification potential of feeds depend on the nature of their carbohydrate fraction, and must be considered for practical applications.

Additional key words: incubation pH; gas production; sugarbeet pulp; sucrose; citrus pulp; wheat bran.

Abbreviations used: ADF (acid detergent fibre); ADL (acid detergent lignin); aNDFom (neutral detergent fibre treated with amylase and excluding residual ashes); BCFA (branched chain fatty acids); BP (sugarbeet pulp); CP (crude protein); CT (citrus pulp); DM (dry matter); DMd (dry matter disappearance); EE (ether extract); MZ (maize grain); OM (organic matter); NDSF (neutral detergent-soluble fibre); SU (sucrose); WB (wheat bran)

Authors' contributions: Conception and design, analysis and interpretation of data: ZA and MF. Performing of experiments: ZA and SY. Drafting of the manuscript: ZA, AV and MF

Citation: Amanzougarene, Z.; Yuste, S.; de Vega, A.; Fondevila, M. (2017). *In vitro* fermentation pattern and acidification potential of different sources of carbohydrates for ruminants given high concentrate diets. Spanish Journal of Agricultural Research, Volume 15, Issue 2, e0602. https://doi.org/10.5424/sjar/2017152-10753

Received: 14 Nov 2016 **Accepted:** 03 May 2017

Funding: MINECO, Spanish Government (project AGL 2013-46820); Department of Industry and Innovation of the Government of Aragón; European Social Fund. SY was granted by the Programa de Formación de Profesorado Universitario (ref. FPU15/01960) from the MECD, Spanish Government.

Competing interests: The authors have declared that no competing interests exist.

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Introduction

The *in vitro* gas production technique (Menke *et al.*, 1979; Theodorou *et al.*, 1994) has been designed for the evaluation of ruminant feeds under optimal fermentation conditions, *i.e.*, at an incubation pH (6.5-6.8) that favours maximum microbial activity. Those systems allow for maintaining incubation pH within a narrow range by adding an important proportion of bicarbonate ion to the incubation solution (Goering & Van Soest, 1970). However, the high rumen fermentability of diets rich in concentrates makes the pH range widely from 6.5 to even 5.5 (Hungate, 1966), clearly modifying rumen population and activity (Russell & Dombrowski, 1980; Hiltner & Dehority, 1983). Besides, concentrate feeds with a rapid rate of fermentation contribute themselves to enhance rumen acidification, but their potential in this sense cannot be evaluated in a strongly buffered medium. Feeds potential of acidification is generally assumed, though to a variable extent, for cereal grains (Chai *et al.,* 2004; Lanzas *et al.,* 2007) and even more for sources of soluble

sugars (Huhtanen & Khalili, 1992; Hoover *et al.*, 2006), but it should also be considered for highly fermentable fibrous feeds, such as citrus pulp or sugarbeet pulp, rich in pectins and fermentable hemicelluloses (Flachowski *et al*., 1993; DePeeters *et al.,* 1997; Barrios-Urdaneta *et al.,* 2003). In addition some cereal byproducts, such as wheat bran, may include important proportions of rapidly available starch that is fermented at a high rate by rumen microbes (Maes & Delcour, 2001).

Conventional incubation conditions with concentrate feeds give higher gas production results because of unrealistic high pH (Fondevila & Pérez-Espés, 2008; Bertipaglia *et al*. 2010), and therefore extrapolation to concentrate feeding is biased by incubation conditions. In previous work in our laboratory, we have modified incubation conditions of the *in vitro* gas production method by reducing the concentration of bicarbonate ion, thus reducing the buffering capacity (Kohn & Dunlap, 1998) and allowing for both the study of fermentation at low medium pH and the study of acidification properties of feeds.

The objective of this study was to evaluate *in vitro* different sources of highly fermentable carbohydrates under two different approaches: at an incubation pH (6.5) that ensures for an optimal rumen microbial fermentation, and at low buffered conditions that allow for the study of both the fermentation of feeds in conditions close to those occurring in high-concentrate feeding systems, and the acidification capacity of such feeds.

Material and methods

Five feeds differing in the nature of their carbohydrate fraction were chosen as substrates for *in vitro* incubation: maize grain (Dekalb 6667YG, MZ); sucrose (SU); wheat bran (WB); sugarbeet pulp (BP) and citrus pulp (CT). All substrates (except for SU) were milled through a sieve of 1 mm using a hammer mill (Retsch Gmbh/SK1/417449). Chemical composition of substrates is given in Table 1.

Four adult Rasa Aragonesa ewes (average weight 70 ± 2.7 kg) fitted with a rumen cannula, were used as donors of rumen inoculum. Animals were housed in the facilities of the Servicio de Experimentación Animal of the Universidad de Zaragoza. Maintenance and extraction procedures of rumen inoculum from donor animals were approved by the Ethics Committee for Animal Experimentation. Care and management of animals agreed with the Spanish Policy for Animal Protection RD 53/2013 (BOE, 2013), which complies with EU Directive 2010/63 (EC, 2010) on the protection of animals used for experimental and other scientific purposes.

Table 1. Chemical composition (g/kg DM) of maize (MZ), wheat bran (WB), sugarbeet pulp (BP) and citrus pulp (CT) incubated as substrates[1].

Chemical composition [2]	MZ	WB	BP	CT
OM	986	944	953	940
CP	75	161	107	59
EE	34	31	5	14
Starch	706	245	—	—
ANDFom	91	499	437	207
ADF	25	145	272	192
ADL	2	37	75	21
NDSF	77	155	457	423
Sugars	13	31	9	243

[1]: It is assumed that sucrose (SU) was 100% soluble sugars. [2]: OM: organic matter; CP: crude protein; EE: ether extract; aNDFom: neutral detergent fibre treated with amylase and excluding residual ashes; ADF: acid detergent fibre; ADL: acid detergent lignin; NDSF: neutral-detergent soluble fibre.

In a first incubation trial, ewes were fed 600 g of alfalfa hay plus 300 g of barley straw for three weeks. Thereafter, in a second incubation trial diet was changed to 500 g of concentrate (0.60 ground barley grain, 0.20 ground maize grain and 0.20 soybean meal) plus 300 g of alfalfa hay for another three weeks, in order to get a rumen environment characteristic of concentrate diets. Along the third week of each period, rumen contents (~ 300 mL) were extracted before the morning feeding, pooled, filtered through cheesecloth and transferred to the laboratory in thermos bottles preheated to 39° C.

First incubation trial: pH 6.5

A first *in vitro* incubation trial was carried out following the procedures of Theodorou *et al.* (1994), in three series of 8 h with four 116 mL glass bottles for each substrate (500 mg of substrate per bottle except for SU, 400 mg). The bottles were filled with 80 mL of incubation solution, which consisted of 0.10 rumen inoculum from ewes given a fibrous (alfalfa-straw) diet and 0.90 of an incubation mixture containing (mL/L): 474 mL distilled water; 238 mL of buffer solution made up with sodium bicarbonate ($NaHCO_3$) and ammonium bicarbonate ($(NH_4)HCO_3$); 238 mL of macro-minerals solution made up with 5.7 g disodium hydrogen phosphate (Na_2HPO_4), 6.2 g potassium di-hydrogen orthophosphate (KH_2PO_4), and 0.6 g magnesium sulphate ($MgSO_4.7H_2O$); and 50 mL of reducing solution made up with 47.5 mL distilled water, 2 mL of 1 N NaOH and 313 mg HCl-cysteine. Micro-minerals solution and resazurin were not included in the incubation medium (Mould *et al.,* 2005a). The

buffer solution included 18.3 g/L of $NaHCO_3$ and 1.9 g/L of $(NH_4)HCO_3$, in order to adjust the incubation pH to 6.50, according to the calculations by Kohn & Dunlap (1998). Ingredients were mixed and allowed to be reduced under a CO_2 atmosphere. Bottles were filled with the incubation solution under a CO_2 stream, sealed with rubber caps and aluminium caps and incubated at 39 ºC. On each incubation run, three additional bottles without substrate were also included as blanks, for subtracting the contribution of inoculum to overall fermentation. Pressure was recorded every 2 h of incubation, by means of an HD 2124.02 manometer fitted with a TP804 pressure gauge (Delta Ohm, Caselle di Selvazzano, Italy). Readings from the two bottles maintained during the whole incubation period were converted into volume by a pre-established linear regression equation between the pressure recorded in the same bottles under the same conditions and known air volumes (n= 103; R^2= 0.996). The gas volume recorded for each incubation time was estimated as the average of the two bottles of each treatment on each series, expressed per unit of incubated organic matter (OM).

One bottle was randomly chosen and opened at 2 and 4 h incubation, and at the end of the incubation (8 h), for measuring incubation pH (CRISON micropH 2001, Barcelona, Spain). Incubation medium after 8 h in the residual bottle was sampled and stored at -20ºC for the analysis of volatile fatty acids (VFA; 2 mL sample, collected over 0.5 mL of a deproteinizing mixture of 0.5M PO_4H_3 with 2 mg/mL 4-methyl valeric acid as internal standard) and lactic acid concentration (2 mL).

Second incubation trial: poorly-buffered medium

In a second approach, the buffer solution included 1.9 g/L of $NaHCO_3$ and 0.1 g/L of $(NH_4)HCO_3$, calculated to establish a pH of 5.50, assumed as a poorly-buffered medium (Amanzougarene & Fondevila, 2017). Three series of incubation of 10 h were carried out, with five bottles per treatment and including inoculum from a concentrate diet, and gas production was measured every 2 h. One bottle per treatment was opened every 2 h, and at the end of the incubation period for measuring pH. The incubation medium from bottles opened at 8 h was sampled for VFA and lactic acid analysis, as above. At the end of the incubation period, bottles were filtered through a 45 μm pore size mesh and dried at 60ºC for 48 h to determine dry matter disappearance (DMd).

Chemical analyses

Substrates were analysed following the procedures of AOAC (2005) for dry matter (DM; reference method 934.01), organic matter (OM; ref. 942.05), crude protein (CP; ref. 976.05) and ether extract (EE; ref. 2003.05) analysis. Concentration of neutral detergent fibre (aNDFom) was analysed as described by Mertens (2002) in an Ankom 200 Fibre Analyser (Ankom Technology, NY), using α-amylase and sodium sulphite, and results are expressed exclusive of residual ashes. The acid detergent fibre (ADF, ref. 973.18) and acid detergent lignin (ADL) were determined as described by AOAC (2005) and Robertson & Van Soest (1981), respectively. Total starch content was determined enzymatically from samples ground to 0.5 mm using a commercial kit (Total Starch Assay Kit K-TSTA 07/11, Megazyme, Bray, Ireland). Neutral detergent-soluble fibre (NDSF) was estimated following Hall *et al.* (1997), discounting the ethanol insoluble EE from the insoluble OM. The solubilized OM fraction was considered as soluble sugars, once corrected for soluble CP and starch.

The frozen samples of incubation media were thawed and centrifuged at 16,000 g for 15 min for their analysis of lactic acid and VFA. The VFA were determined by gas chromatography on an Agilent 6890, apparatus equipped with a capillary column (HP-FFAP polyethylene glycol TPA, 30 m × 530 μm Id). Isobutyrate and isovalerate are considered together as branched-chain fatty acids (BCFA). The lactic acid concentration was determined by the colorimetric method proposed by Barker & Summerson (1941).

Statistical analysis

Results were analysed by ANOVA using the Statistix 10 software package (Analytical Software, 2010). For both incubation trials, the effect of the cereal sources on pH, *in vitro* gas production, total VFA concentration, VFA profile and lactic acid concentration was studied for each time of incubation, considering the incubation series as a block. The differences were considered significant when $p<0.05$, and a trend for significance was considered when $0.05<p<0.10$. The Tukey *t* test ($p<0.05$) was used for the multiple comparison between means.

Results

First incubation trial

The pH of the inoculum when obtained from the donor sheep was 7.0 ± 0.14. As planned, incubation pH from 2 to 8 h of incubation was fairly constant, ranging between 6.3 and 6.6. Despite a higher pH ($p<0.05$) was recorded at all incubation times with the extreme treatments MZ (values from 6.54 to 6.59) and CT (from 6.45 to 6.33), treatment differences were lower than

0.3 units throughout the incubation period. There was a trend (p=0.052) for a higher gas production with CT than with SU, WB and MZ at 2 h of incubation (Fig. 1), whereas volume with CT was higher (p<0.05) than with MZ from 4 h onwards, SU from 6 h onwards, and BP at 8 h of incubation. No differences were recorded among the other substrates. In the same sense, no differences (p>0.05) among substrates were recorded on total VFA concentration, or on molar proportions of acetate and propionate when incubated at pH 6.5 (Table 2). However, proportions of butyrate and BCFA were lowest (p<0.05) with CT and BP, and that of valerate was also lowest for these and SU (p<0.05). No substrate differences were recorded on lactic acid concentration (p>0.05) at 8 h of incubation, partly because of the large magnitude of the error term, all substrates showing a low magnitude ranging from 0.2 to 2.5 mM.

Second incubation trial

Rumen inoculum pH was 6.4 ± 0.04. Pattern of incubation pH in a poorly buffered medium (Fig. 2) shows a rapid drop at 2 h of incubation, reaching an average value of 5.76, and then a gradual fall until 10 h of incubation for WB, BP and MZ (final values of 5.31 to 5.28), with a higher magnitude for CT (final pH 5.08). However, SU dropped from 4 h onwards, reaching the lowest (p<0.05) values and a final pH of 4.00 at 10 h of incubation.

Throughout the incubation period, CT produced more gas (p<0.05) than BP and MZ, and more than SU and WB from 4 and 6 h, respectively, whereas SU and WB were also higher than BP and MZ at 4, 6, 8 and 10 h of incubation (Fig. 3). Volume of gas with MZ and BP did not differ from that with SU at 10 h of incubation (p>0.05). The proportion of DMd at the end of the 10 h incubation period was almost complete with SU (0.981), whereas CT and WB recorded higher (p<0.05) values than MZ and BP (0.411, 0.367, 0.246 and 0.231, respectively; SEM= 0.0146).

Total concentration of VFA (Table 3) after 8 h of incubation was higher with CT than with BP, SU and MZ (p<0.05). Molar proportion of acetate was higher, and that of propionate lower, with BP than WB (p<0.05), whereas butyrate was higher with MZ and WB than with BP and CT (p<0.05). Proportion of BCFA was higher with MZ and WB than CT, being also higher with MZ than with SU and BP. Lactic acid concentration was higher (p<0.05) with SU than WB and BP, whereas CT and MZ recorded intermediate values.

Discussion

The studied substrates were chosen based on the varied chemical composition of their carbohydrate fraction (Table 1). Maize is a model of cereal grain, with a high proportion of starch, although it is available in the gut at a relative moderate rate compared to other grains (Offner *et al*., 2003; Lanzas *et al*., 2007). As a byproduct of cereal processing, wheat bran includes a variable but important proportion of starch (100 to 240 g/kg), highly available since it is from wheat, and its cell wall contains a major proportion of fermentable hemicelluloses (Maes & Delcour, 2001; FEDNA, 2010). Sugarbeet pulp has high proportions of both neutral detergent fibre (Fondevila *et al*., 1994; FEDNA, 2010) and soluble fibre, mostly pectins, that can be fermented at a similar rate than soluble starch (Fondevila *et al*., 2002), whereas CT is a source of both soluble fibre and soluble sugars (Hall, 2002; Barrios-Urdaneta *et al*., 2003). Finally, SU was chosen as a model for soluble sugars that are almost completely used by most rumen microbes in less than an hour (Hristov *et al*., 2005).

As a reference, in Experiment 1 substrates were compared under conventional incubation conditions, with inoculum from sheep given a forage diet,

Table 2. Total concentration of volatile fatty acids (VFA, mM) and molar VFA proportions, together with lactic acid concentration (mM), recorded from maize (MZ), sucrose (SU), wheat bran (WB), sugarbeet pulp (BP) and citrus pulp (CT) after 8 h of *in vitro* incubation at pH 6.5 (first trial).

	MZ	SU	WB	BP	CT	SEM[1]
VFA	13.7	16.9	22.9	22.3	21.7	3.68
Acetate	0.539	0.524	0.497	0.545	0.551	0.0226
Propionate	0.188	0.216	0.232	0.211	0.203	0.0213
Butyrate	0.053a	0.049a	0.052a	0.056b	0.057b	0.0040
Valerate	0.004ab	0.003b	0.006a	0.003b	0.003b	0.0006
BCFA[2]	0.014a	0.007ab	0.013ab	0.005b	0.005b	0.0019
Lactic acid	0.30	0.52	0.19	2.27	2.49	1.604

[1]: SEM: standard error of the means. [2]: BCFA: sum of isobutyrate and isovalerate proportions. Within rows, different letters show differences among means (p<0.05)

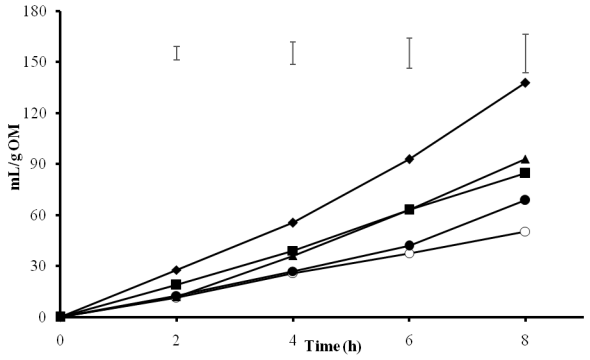

Figure 1. Gas production (mL/g organic matter, OM) from maize grain (○), sucrose (●), wheat bran (▲), sugarbeet pulp (■) and citrus pulp (◆) when incubated *in vitro* at a pH 6.5 (first trial). Upper bars show the standard error of the means.

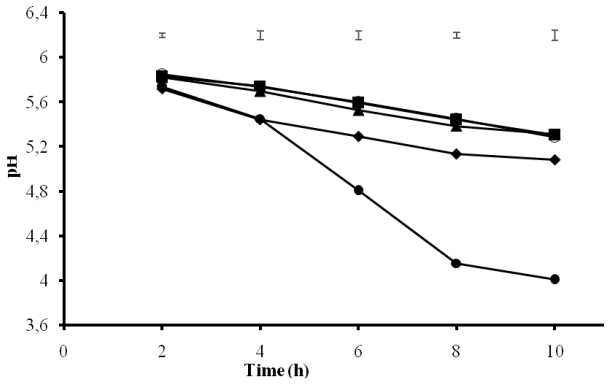

Figure 2. Pattern of incubation pH from maize grain (○), sucrose (●), wheat bran (▲), sugarbeet pulp (■) and citrus pulp (◆) when incubated *in vitro* in a poorly-buffered medium (second trial). Upper bars show the standard error of the means.

and maintaining incubation pH within a range that should allow for maximising potential fermentation. According to the volume of gas produced *in vitro*, CT in Experiment 1 was fermented at the highest extent during the 8 h incubation, but this was not manifested in higher total VFA concentration, partly because of the high magnitude of the error term (coefficient of variation 0.19), nor in the VFA pattern except for a higher butyrate molar proportion with the substrates rich in soluble fibre (CT and BP). Despite of differences in the nature of their carbohydrate fractions, gas production from BP and WB evolved similarly throughout the 8 h incubation period, whereas values at 6 and 8 h for the two non-fibrous substrates SU and MZ were lower than the other under these incubation conditions, probably because the inoculum induced by a forage diet, such as in Experiment 1, was less specialised in fermenting sugars or starch than that from a concentrate diet (Mould *et al*., 2005b).

In any case, Experiment 1 was carried out as a standard reference, but the major interest of the present work was to compare substrates in incubation conditions close to those found with concentrate-rich diets, such in Experiment 2, where the inclusion of an inoculum from a concentrate diet and the low buffering potential of the incubation solution make the procedure closer to natural incubation conditions in intensive feeding. In this case, acidification caused by fermentation of substrates incubated in a medium with a low buffering capacity did not differ between MZ, BP and WB, pH being reduced in 1.2 units from that of inoculum after 10 h of incubation, with most of this response occurring in the first 2 h (Fig. 2). The magnitude of this drop increased to 1.4 pH units when CT was incubated, reaching a final pH of 5.08 after 10 h of incubation. For SU, the buffering capacity of the medium was exhausted from 4 h onwards, and pH fell from 5.45 to 4.00 at 10 h because of its high rate and extent of fermentation. This should probably be linked to the high concentration of lactic acid recorded at 8 h incubation, in agreement with that reported by Strobel & Russell (1986). Therefore, the inclusion of high levels of sucrose sucrose in concentrate diets may contribute to enhance the risk of acidosis, because of its fast fermentation rate, thus reducing the potential microbial activity over this and the whole diet. In fact, fermentation was almost stopped from 6 h onwards, probably because of the worsening of incubation conditions. These results make recommend a low level of sugar inclusion in ruminant diets (Vallimont *et al.,* 2004).

If gas production volumes observed after 8 h incubation in both experiments are contrasted, fermentation of CT in Experiment 2 (Fig. 3) was proportionally reduced in 0.29 with respect to that

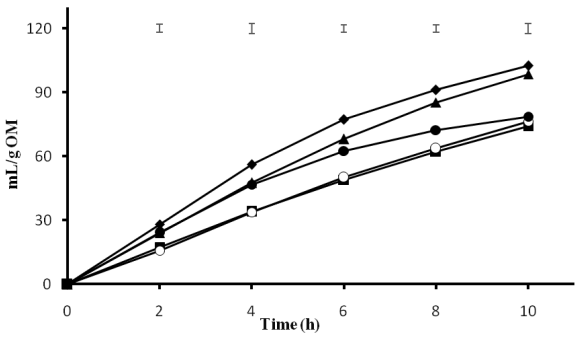

Figure 3. Gas production (mL/g organic matter, OM) from maize grain (○), sucrose (●), wheat bran (▲), sugarbeet pulp (■) and citrus pulp (◆) when incubated *in vitro* in a poorly-buffered medium (second trial). Upper bars show the standard error of the means.

Table 3. Total concentration of volatile fatty acids (VFA, mM) and molar VFA proportions, together with lactic acid concentration (mM), recorded from maize (MZ), sucrose (SU), wheat bran (WB), sugarbeet pulp (BP) and citrus pulp (CT) after 8 h of *in vitro* incubation in a poorly-buffered medium (second trial).

	MZ	SU	WB	BP	CT	SEM[1]
VFA	27.4b	30.0b	33.8ab	30.5b	38.5a	2.01
Acetate	0.472ab	0.473ab	0.437b	0.509a	0.494ab	0.0190
Propionate	0.185ab	0.193ab	0.222a	0.177b	0.193ab	0.0121
Butyrate	0.113a	0.109ab	0.112a	0.090b	0.090b	0.0060
Valerate	0.006	0.006	0.006	0.006	0.006	0.0004
BCFA[2]	0.024a	0.019bc	0.023ab	0.018bc	0.015c	0.0014
Lactic acid	2.87ab	12.70a	0.42b	0.18b	3.10ab	0.708

[1]: SEM: standard error of the means. [2]: BCFA: sum of isobutyrate and isovalerate proportions. Within rows, different letters show differences among means ($p<0.05$)

recorded in Experiment 1 (Fig. 1). In Experiment 2, incubation pH with this substrate dropped to 4.8 after 6 h incubation, and continued falling afterwards (Fig. 2). This should explain why rate of gas production from CT was reduced from 4 h onwards in Experiment 2, instead of continuing increasing like in Experiment 1. The high content in both soluble sugars and soluble fibre of CT (Table 1) should promote a high rate of fermentation that was also manifested on a high VFA concentration (Table 3), thus leading to a low incubation pH. These altered conditions should prevent from a higher DMd for this substrate. It can be considered that, since incubation conditions became unfavourable for microbial activity after 6 h, fermentation of citrus pulp was uncompleted, mostly affecting the fibrous fraction since fibrolytic activity is minimal before that time (Fondevila *et al.,* 2002; Mould *et al.,* 2005a). In this regard, straw diets for sheep supplemented with 0.42 citrus pulp did not apparently behaved differently *in vivo* than those with the same proportion of barley grain, with a minimum pH of 5.8 after 4 h (Barrios-Urdaneta *et al.*, 2003). These results, however, cannot be directly extrapolated to practical feeding conditions, since citrus pulp composition may widely vary among batches. In this sense, from 79 batches of this by-product, Hall (2002) reported that proportions of soluble sugars and soluble fibre varied from 125 to 402 and from 252 to 437 g/kg DM, respectively.

When comparing results from both experiments, it should be considered that gas production in Experiment 2 could be reduced to a certain extent by a lower contribution of indirect gas coming from the buffering of produced VFA (Beuvink & Spoelstra, 1992) because of the reduced concentration of bicarbonate ion in the poorly-buffered medium. Besides, drop of pH as incubation proceeds was higher with the inoculum from a concentrate diet (Experiment 2), negatively affecting microbial community, in a higher extent to fibrolytic bacteria (Russell & Dombrowski, 1980). This should partly explain why gas production from BP at 8 h of incubation was proportionally 0.27 lower in Experiment 2 than in Experiment 1, mainly considering that rumen microbes from concentrate-rich conditions are less capable to ferment fibre than those from a forage diet, as suggested by Calsamiglia *et al*. (2008). In contrast to gas production results, total VFA concentration was higher in Experiment 2, but this may be explained by the self-fermentation of the inoculum itself, which harbours a higher concentration of soluble nutrients, whereas gas production results were corrected in both experiments by the volume of gas produced from the blanks of inoculum (incubated without substrate), thus minimising such bias.

In contrast, fermentation of MZ increased in a proportion of 0.28 under conditions of Experiment 2, indicating that rumen microbial population promoted by forage diets is not adapted to ferment the moderately available vitreous starch of maize (Nagadi *et al.,* 2000). Finally, values observed with WB and SU were affected to a minor extent by incubation conditions (0.09 proportionally lower and 0.04 higher gas volume in Experiment 2 than 1, respectively). The mixed characteristics as a source of fermentable fibre but with a noticeable proportion of highly fermentable starch (Lanzas *et al.*, 2007) in case of WB, and the ease for a rapid utilization of mono- and disaccharides for most rumen microorganisms in SU (Hristov *et al.,* 2005) should explain those minor differences.

In conclusion, the extent and rate of fermentation of sugars and soluble fibre fractions from citrus pulp are of a higher magnitude than those from other carbohydrates, such as the highly available starch and hemicellulose from wheat bran. In contrast, starch from other sources like maize grain is fermented at a slower rate, similar to that of the cell wall and soluble fibre of sugarbeet pulp. These last three feeds contribute at a minor extent to acidification of the medium, and therefore their impact on microbial fermentation should be lower.

In contrast, sucrose as a source of soluble sugars has a high acidification potential, which may reduce its potential fermentation from 4 h onwards by challenging the microbial activity when include at a high level. The practical implications of these responses, in order to choose the level of each carbohydrate source in high-concentrate diets will depend on the objectives and constraints of the feeding system itself.

Compared to conventional *in vitro* incubation conditions, a poorly-buffered medium allows for the study of fermentation of substrates differing in the nature of their carbohydrate fraction, when used in high-concentrate diets.

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