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RESEARCH ARTICLE

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In vitro characterisation of the rumen fermentation pattern of the cell wall fraction from several fibrous sources

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Abstract

Aim of study: To isolate fibre effect from other factors when comparing fibrous sources, the rumen fermentation pattern of extracted cell walls was studied.

Material and methods: Cell wall fractions from soybean hulls (SH), sugarbeet pulp (BP), palm kernel cake (PK), oat hulls (OH), dehydrated alfalfa meal (DA) and barley straw (BS) were incubated in four 48 h series.

Main results: Cell wall extraction efficiency was ± 0.07 units over the neutral detergent fibre content, except for PK, which recovery was 0.20. Gas produced from BP and SH was higher (p<0.05) from 6 h. PK behaved similarly to SH from 6 to 24 h but maintained constant thereafter, whereas gas volume from OH was the lowest from 24 to 48 h (p<0.05). All substrates recorded a maximum rate of gas production at 12 h, except OH, for which fermentation was constant on time. The organic matter disappearance after 48 h incubation agreed with these results, being higher with BP and SH, whereas OH was the lowest (p<0.05). The proportion of methane in total gas produced was higher in OH than BP at 36 and 48 h (p<0.05). The highest total VFA concentration was recorded with BP (p<0.05). Propionate proportion was enhanced from BP, BS and SH, and that of butyrate was higher with PK and OH, whereas no differences among substrates were recorded in acetate proportion.

Research highlights: Fermentation of the cell wall fraction of fibrous feeds is not directly linked to its chemical composition, not even to its lignin proportion.

Additional key words: neutral detergent fibre; gas production; non-forage fibrous sources.

Abbreviations used: ADFom (acid detergent fibre, excluding of residual ashes); BCVFA (branched-chain volatile fatty acids); BP (sugarbeet pulp); BS (barley straw); DA (dehydrated alfalfa meal); DM (dry matter); NDF (neutral detergent fibre, including ashes); NDFom (neutral detergent fibre, excluding of residual ashes); OH (oat hulls); OM (organic matter); OMd (organic matter disappearance); PK (palm kernel meal); SH (soybean hulls); VFA (volatile fatty acids.

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Introduction

There is an increasing interest for using non-forage fibre sources, often agricultural by-products, in diets for ruminant feeding, as they can contribute to adjust feed costs compared with forages (Bradford & Mullins, 2012). These feeds are generally included as ingredients in the compound feed, and although non-forage fibre based diets can maintain or even improve rumen health and performance of dairy cattle under certain conditions (Pereira *et al.*, 1999; Ertl *et al.*, 2015), their use as main sources of fibre is controversial. Their particle size is smaller

compared with forages, thus promoting a limited effect as potentially effective fibre, which is necessary for stimulating rumination and modulating rumen pH in high concentrate feeding conditions (Armentano & Pereira, 1997; Grant, 1997).

Because of their variable origin, the composition of non-forage fibre sources is very heterogeneous. Their fibrous fraction can include high proportions of cell wall, such as insoluble polysaccharides (cellulose or hemicelluloses), like in seed hulls or crop and harvest wastes (Hsu *et al.*, 1987; Gasa *et al.*, 1989; DePeeters *et al.*, 1997), as well as of pectin and soluble fibre, like in sugarbeet pulp or citrus pulp (Bampidis & Robinson, 2006; Münnich *et al.*, 2017). Besides, the lignin content in some fibrous feeds may be important. Composition also varies widely in their non-fibrous fraction, with different proportions of protein or even lipids among sources.

The amount, composition and structure of the fibrous fraction of feeds determines the rate and extent of their rumen fermentation (Ford & Elliot, 1989; Chesson, 1993; Miron *et al.*, 2001), in terms of microbial access and activity (Krause *et al.*, 2003; Wang *et al.*, 2018). Further, lignin forms complexes with cellulose and hemicelluloses and affects fermentation of structural polysaccharides (Chesson, 1993; Jung & Deetz, 1993; Wilson, 1994). In addition, other nutrient compounds interact with the fibrous fraction, and must also be considered in order to compare their nutritive value as fibre sources. Thus, released fatty acids from lipolysis may negatively affect microbial activity and thus reduce degradation (Doreau & Ferlay, 1995; Beauchemin *et al.*, 2009). Besides, protein proportion is inversely related to feed fermentation, partly because it is less fermentable than that of carbohydrates (Getachew *et al.*, 1997) but also because the released ammonia binds to the CO_2 produced, underestimating fermentation if measured by gas production (Cone & Van Gelder, 1999). Thus, comparing fibrous substrates without interactions with other feed nutrients that may interfere in their response when included in ruminant diets seems necessary for giving a clear approach to their value as fibre sources. Therefore, this work aimed to study, under *in vitro* conditions, the rumen fermentation pattern of the cell wall fraction extracted from several forage and non-forage fibrous sources. A previous evaluation of the same non-extracted substrates has been previously published by Ortolani *et al.* (2020).

Material and methods

Substrates and inocula

The substrates chosen for this comparative study were: soybean hulls (SH), sugarbeet pulp (BP), palm kernel cake (PK), oat hulls (OH), dehydrated alfalfa meal (DA) and barley straw (BS). Chemical composition is given in Table 1. Substrates were ground in a hammer mill (Retsch Gmbh/SK1/417449, Haan, Germany) through a sieve of 1 mm. Cell wall was extracted from ground substrates following the procedure by Smith & Waldo (1969), after boiling in neutral detergent solution (100 mL/g substrate) for 75 min. Residues were extensively washed with distilled water and fixed with acetone, dried and stored until their use as incubation substrates.

Rumen fluid as inoculum was extracted from four adult ewes (54.5 ± 6.8 kg live weight) housed in the facilities of the Servicio de Apoyo a la Experimentación Animal of the Universidad de Zaragoza. Donor animals were fed on 1 kg of a mixed diet composed of (g/kg): alfalfa hay 250; barley straw 250; barley 300; maize 100; soybean meal 100, in a single daily offer at 09:00, from three weeks before the experiment. Before feeding, rumen contents (approximately 300 mL) of each animal were sampled and filtered through a cheesecloth, mixed, collected in thermos flasks and immediately transferred to the lab for incubation. Animal care and procedures for extraction of rumen inoculum were approved by the Ethics Committee for Animal Experimentation (protocol PI48/20). 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 (EU, 2010) on the protection of animals used for experimental and other scientific purposes.

Experimental procedures

Four incubation runs (48 h) were carried out, in a water bath at 39ºC. Incubation procedures were according to Theodorou *et al.* (1994) procedures, but without microminerals and resazurin (Mould *et al.*, 2005). Concentration

Table 1. Chemical composition (g/kg DM) of original feeds (SH, soybean hulls; BP, sugarbeet pulp; PK, palm kernel meal; OH, oat hulls; DA, dehydrated alfalfa meal; BS, barley straw) before cell wall extraction.

	SH	BP	PK	OH	DA	BS
Organic matter	946	920	962	953	886	872
Crude protein	179	85	157	48	133	61
Ether extract	18	3	88	11	18	13
NDF	594	456	616	778	532	763
NDFom	592	442	555	771	513	760
ADFom	417	234	352	376	343	434
Lignin (sa)	16	23	87	56	66	40

DM, dry matter; NDF: neutral detergent fibre (including ashes); NDFom: neutral detergent fibre (excluding ashes); ADFom: acid detergent fibre (excluding ashes); Lignin (sa): lignin.

of bicarbonate buffer in the incubation solution was adjusted as in Amanzougarene & Fondevila (2018) to get a medium pH of 6.5. A total of 28 bottles per run were incubated, with 4 bottles per treatment plus another 4 bottles without substrate considered as blanks of inoculum. Internal pressure of two bottles per treatment was recorded at 2, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h for determination of gas production, and the gas was released. Thereafter, these bottles were opened, and their pH measured (CRISON micropH 2001, Barcelona, Spain) to validate the incubation conditions, and the whole incubation content was filtered through nylon bags (45 µm pore size) that were dried at 60ºC for 48 h to determine organic matter disappearance (OMd).

Concentration of methane in the gas produced was determined at successive time intervals along the incubation (0 to 12 h, 12 to 24h, 24 to 36 h and 36 to 48 h), by taking a single sample (5 mL) of the gas produced in each interval. Gas samples for 0 to 12 h and for 12 to 24 h were collected from the third and fourth incubated bottles per treatment, respectively, whereas one of the two bottles used for gas production was used for gas sampling in the 24 to 36 h and 36 to 48 h intervals, the same bottle for both periods. For each substrate and incubation series, methane concentration was extrapolated to average total gas for each time interval to calculate total methane production per unit of incubated substrate. Two milliliters of liquid medium from the bottles incubated for 12 h was also sampled on 0.5 mL solution of 0.5M phosphoric acid with 1 mg of 4-methyl-valeric acid as internal standard, and was stored at -20ºC for the analysis of volatile fatty acid (VFA) concentration.

Analytical procedures

Substrates were analysed for dry matter (DM), organic matter (OM), crude protein and ether extract content according to the AOAC (2005) procedures (methods ref. 934.01, 942.05, 976.05 and 2003.05, respectively). Their concentration in neutral detergent fibre was analysed as described by Mertens (2002) in an Ankom 200 Fibre Analyser (Ankom Technology, New York), using α–amylase and sodium sulphite, and results being expressed both including (NDF) or excluding (NDFom) residual ashes. The acid detergent fibre, expressed exclusive of residual ashes (ADFom, ref. 973.18) and lignin determined with sulphuric acid were analysed as described by AOAC (2005) and Robertson & Van Soest (1981), respectively. Incubation residues were also analysed for t heir DM and OM content.

Internal pressure on each bottle was measured with a HD8804 manometer provided with a TP804 pressure gauge (DELTA OHM, Caselle di Selvazzano, Italy). Readings were corrected for the atmospheric pressure and converted to volume (mL) using a pre-established linear regression (n=103, R^2=0.996) recorded in the same type of bottles and expressed per unit of incubated OM. Results are presented either as accumulated gas volumes (total gas produced after a given period of time) or as a rate of gas production (volume of gas per unit of time produced in a specific interval). Methane concentration in gas samples was measured in an Agilent 6890 apparatus (Agilent Technologies España, Madrid) equipped with a capillary column (HP-FFAP polyethylene glycol TPA, 30 m × 530 µm id), calibrated with a 10% CH_4 standard, with a flux of 2 mL/min at 250 ºC. The frozen samples of the incubation medium were thawed and centrifuged at 13,000 g for 15 minutes at 4 ºC for their analysis of VFA, that were determined by gas chromatography on the same apparatus than for methane analysis.

Calculations and statistical analyses

Results were analysed statistically by ANOVA using the Statistix 10 package (Analytical Software, 2010), considering the substrate (n=6) as factor and the incubation series (n=4) as a block. For total gas production and OMd, the experimental unit was the average of the two bottles per treatment incubated for 48 h in the same run, whereas for methane production (from 12 to 48 h) and VFA pattern at 12 h the value from a single bottle per run was considered. Treatment differences among means with $p<0.05$ and $0.05<p<0.10$ were accepted as representing statistically significant differences and a trend to differences, respectively. When significant, differences were contrasted by the Tukey test.

Results

Cell wall recovery from SH, BP, PK, OH, DA and BS, measured as weight of residue after large scale extraction with neutral detergent, was 586, 478, 669, 774, 534 and 707 g/kg DM of original feeds, respectively. Initial incubation pH (0 h) averaged 6.36 ± 0.20 for the four incubation runs. Along the whole incubation period, pH values were maintained within a narrow range (from 6.38 to 6.66), not existing differences over 0.15 pH units among substrates at any time interval (12, 24 and 48 h) or over 0.25 units among times within each substrate.

The pattern of total accumulated gas production (Fig. 1) showed substrate differences along the whole incubation period ($p<0.01$). From 6 h onwards cell walls from BP and SH showed higher volumes of gas ($p<0.05$), although PK behaved similarly to SH from 6 to 24 h and recorded minimum increases thereafter. Fermentation of the other three substrates did not greatly differ from PK

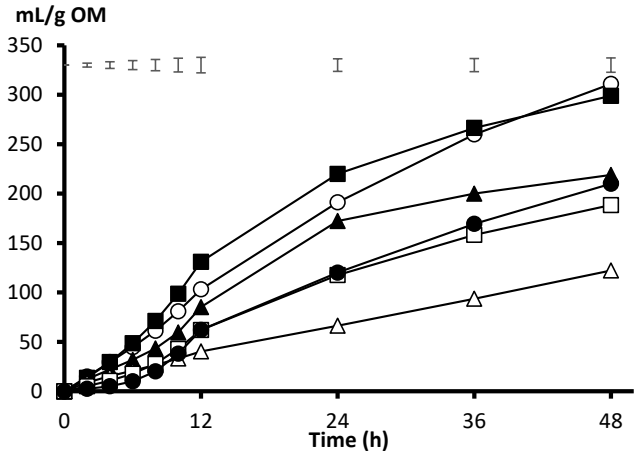

Figure 1. *In vitro* gas production pattern (mL/g OM) of cell wall fractions extracted from soybean hulls (◯), sugarbeet pulp (■), palm kernel meal (▲), oat hulls (△), dehydrated alfalfa hay (□) and barley straw (●). Upper bars show standard error of means.

up to 12 h incubation, and at 48 h differences between this and DA and BS again became non-significant. Gas production from OH was the lowest from 24 to 48 h (p<0.05). For a better characterisation of cell wall fermentation, the rate of gas production as volume per time unit is presented in Fig. 2. All substrates reached a maximum gas production at 12 h, except for OH, which rate was relatively constant along the whole incubation, ranging from 2.2 to 4.5 mL/g OM/h. This and the moderate magnitude of increases from the initial 2 h to the maximum for SH (from 7.4 to 11.1 mL/g OM/h) contrast with changes observed with BP, PK and DA (9.4, 6.6 and 6.5 mL/g OM increase)

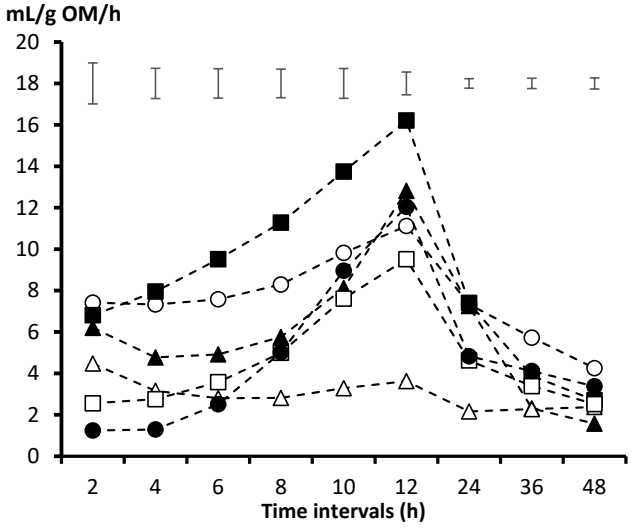

Figure 2. Rate of *in vitro* gas production pattern (mL/g OM per h) of cell wall fractions extracted from soybean hulls (◯), sugarbeet pulp (■), palm kernel meal (▲), oat hulls (△), dehydrated alfalfa hay (□) and barley straw (●). Upper bars show standard error of means.

and mainly the 9.7-fold increase (from 1.2 to 12.0 mL/g OM/h) in BS in the same period. Rate of fermentation of BP was the highest from 6 to 12 h and that of OM was the lowest from 10 to 24 h (p<0.05), being the comparison among the other substrates similar to that commented regarding Fig. 1.

Proportion of methane in the gas produced at the different incubation time intervals (Table 2) showed differences only at the end of incubation (36 to 48 h), when methane proportion from fermentation of OH was higher than that with BP, not existing differences among the rest of substrates. When the volume of methane produced was expressed per unit of incubated substrate (Fig. 3), the production with OH was lower than BP, SH and DA from 0 to 12 h, and was the lowest from 24 to 48 h (p<0.05). Higher methane production was observed with SH, BP and PK at 24 h, but differences among PK, DA and BS became non-significant at 36 h. After 48 h of incubation, substrates ranked as follows: SH>BP, PK, DA, BS > OH (p<0.05). The OMd after 48 h incubation (Table 2) was higher with BP and SH, followed by PK and BS and then DA, whereas OH was the lowest (p<0.05).

Total VFA concentration and molar proportions of the major VFAs after 12 h incubation are presented in Table 3. The highest total VFA concentration was recorded with BP, and it was also higher with DA than OH, recording intermediate values with the rest of substrates (p<0.05). No substrate differences were recorded on acetate proportion, whereas that of propionate was higher with BP than PK, OH and DA, and with SH and BS it was higher than PK (p<0.05). Instead, butyrate proportion was higher with PK and OH than BS, and higher in SH than BP and DA. OH recorded higher valerate and branched-chain volatile fatty acids (BCVFA, sum of isobutyrate and isovalerate) proportions than SH and DA (p<0.05).

Discussion

Compared with the analysed NDF proportion of substrates (without discounting ashes) that is shown in Table 1, the proportion of cell wall recovery in the extraction process was 0.99, 1.05, 1.09, 0.99, 1.00 and 0.93, respectively. Extraction efficiency was within ± 0.09 units interval, with extreme values for BS and PK. In the former substrate, the lower recovery could be associated to a partial solubilisation of cell wall when processed at a larger scale, as it was also observed by Barrios Urdaneta *et al.* (2000). In the case of PK, the difference could be due to a noticeable proportion of cracked stones in this by-product that might bias the NDF proportion in the reference analysis from this substrate because of problems in sampling, aspect that should be minimised when a higher initial

Table 2. Methane (proportion of total gas) produced for the different substrates (soybean hulls, SH; sugarbeet pulp, BP; palm kernel meal, PK; oat hulls, OH; dehydrated alfalfa hay, DA; barley straw, BS) incubated at successive time intervals, together with organic matter disappearance (OMd) after 48 h.

Substrates	0-12 h	12-24 h	24-36 h	36-48 h	OMd
SH	0.060	0.174	0.153	0.160^{ab}	0.816^{a}
BP	0.052	0.166	0.148	0.159^{b}	0.829^{a}
PK	0.051	0.192	0.152	0.163^{ab}	0.650^{b}
OH	0.043	0.166	0.164	0.179^{a}	0.240^{d}
DA	0.076	0.178	0.165	0.169^{ab}	0.493^{c}
BS	0.048	0.153	0.169	0.161^{ab}	0.650^{b}
SEM	0.0100	0.0137	0.0061	0.0042	0.0132
p-value	0.29	0.48	0.14	0.026	<0.001

SEM: standard error of means. Within columns, letters indicate significant differences (p<0.05)

amount of substrate was used for the large batch extraction respect to chemical determination.

In order to validate the process of cell wall isolation in this experiment, it is assumed that extraction accurately reflects the entire cell wall fraction of all sources, and thus allowed for a homogeneous comparison of their fermentation, without interferences from other components of the original feed (Barrios Urdaneta *et al.*, 2000; Zhang *et al.*, 2007). In this regard, it is worth considering that chemical analysis of substrates showed a crude fat content of 88 g/kg DM in PK respect to a range from 3 to 18 g/kg in the other substrates, and a crude protein content of 179, 157 and 133 g/kg DM in SH, PK and DA *vs.* a range from 48 to 85 g/kg in BP, BS and OH (Table 1).

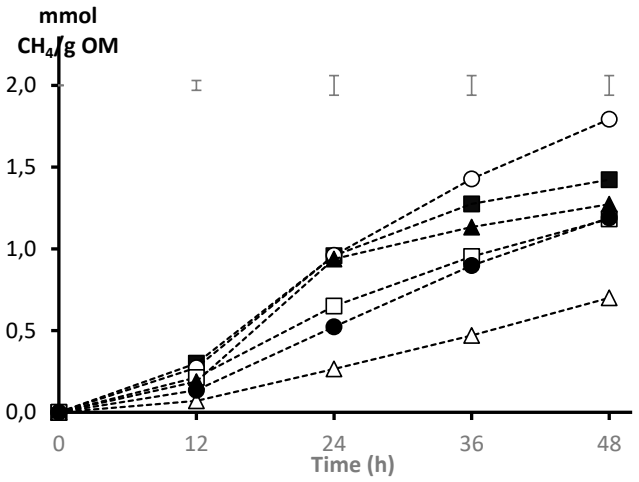

Figure 3. Methane production pattern (mmol/g OM) of cell wall fractions extracted from soybean hulls (◯), sugarbeet pulp (■), palm kernel meal (▲), oat hulls (△), dehydrated alfalfa hay (□) and barley straw (●). Upper bars show standard error of means.

In other way, some cell wall components such as pectins and to some extent non-lignified xylans are solubilised by neutral detergent (Van Soest, 1994; Jung & Allen, 1995) and thus were not recovered when processed for cell wall extraction. Therefore, fermentation potential of non-processed BP, which contains from 13 to 20% pectins (Miron *et al.*, 2001; FEDNA, 2019) might be underestimated in substrate comparison. In fact, cell wall fermentation measured as gas production after 48 h incubation was 0.86 of that of the whole ingredient of the same batch reported by Ortolani *et al.* (2020). Other substrates such as SH and DA may also contain noticeable proportions of pectins (around 8 % in both SH and DA; FEDNA, 2012), and thus a reduction in fermentation could be expected when this fraction was removed. However, this might be counterbalanced by the protein washout in the cell wall treatment, as nitrogen content is negatively correlated with gas production (González-Ronquillo *et al.*, 1998; Cone & Van Gelder, 1999). Thus, despite the 179 and 133 g CP/kg DM of SH and DA (Table 1), fermentation of their extracted cell wall fractions resulted in gas volumes after 48 h close to those reported with the original substrate (Ortolani *et al.*, 2020).

Cell wall polysaccharides from BP and SH were rapidly and extensively fermented, at a slower but more constant rate for the latter (Fig. 2), thus making that extent of gas production in the first 24 h interval was 0.74 *vs.* 0.61 of total gas with BP *vs.* SH, although both of them reached similar gas volume after 48 h (Fig. 1). Getachew *et al.* (2004) observed a 0.76 NDF digestibility after 24 h of *in vitro* incubation. In both cases, such pattern could be related with its high availability of cell wall polysaccharides (Miron *et al.*, 2001; Seo *et al.*, 2009) and low lignin content (0.03 - 0.05 of total NDF). Initially, rate of gas production for PK was relatively constant from 2 to

Table 3. Total volatile fatty acid (VFA) concentration (mM) and molar proportions (%) of VFA with the different substrates (soybean hulls, SH; sugarbeet pulp, BP; palm kernel meal, PK; oat hulls, OH; dehydrated alfalfa hay, DA; barley straw, BS) at 12 h of incubation.

Substrates	Total VFA	Acetate	Propionate	Butyrate	Valerate	BCVFA
SH	30.37^{bc}	60.09	20.71^{ab}	15.07^{ab}	0.79^{b}	3.34^{bc}
BP	44.75^{a}	61.33	23.51^{a}	12.17^{c}	0.66^{b}	2.34^{c}
PK	31.83^{bc}	61.02	16.82^{c}	17.19^{a}	0.86^{ab}	4.12^{c}
OH	24.86^{c}	58.45	18.81^{bc}	16.23^{a}	1.09^{a}	5.43^{a}
DA	34.45^{b}	64.19	19.14^{bc}	12.46^{c}	0.76^{b}	3.46^{bc}
BS	29.89^{bc}	60.71	21.35^{ab}	13.21^{bc}	0.86^{ab}	3.89^{b}
SEM	1.904	1.269	0.655	0.524	0.056	0.270
p-value	<0.001	0.11	<0.001	<0.001	<0.01	<0.001

SEM: standard error of means; BCVFA: branched chain VFA (sum of isobutyrate and isovalerate). Within columns, letters indicate significant differences ($p<0.05$))

8 h and then increased up to 12 h, but fermentation from 24 h onwards was notably diminished, and only 0.21 of total gas production was produced thereafter. It seems that availability of fermentable polysaccharides was rapidly reduced, and the masking effect of its high lignin content (0.16 of total NDF) over polysaccharides probably was then more apparent. According to results from Hindle *et al.* (1995), the proportion of rumen undegradable fraction in palm kernel cake may reach up to 0.37 of total NDF. Non-processed OH had a high cell wall content (Table 1), but in the range previously reported (Garleb *et al.*, 1991; Thompson *et al.*, 2000). The low rate and extent of cell wall fermentation of OH, even lower than that from straw, agrees with previous in situ results (Hsu *et al.*, 1987; Thompson *et al.*, 2000), although it may widely vary depending on the oat variety. The low fermentation of OH can be attributed to its high lignin content and the presence of tight lignin/carbohydrate complexes (Garleb *et al.*, 1991). However, for a similar feed such as wheat bran, Miron *et al.* (2001) suggest that it is cutin (also recovered in the lignin analysis) rather than lignin which restricts microbial fermentation, and Jung & Allen (1995) indicate that determination of lignin as soluble in sulphuric acid tends to underestimate lignin content of grasses.

According to Miron *et al.* (2001), cell walls from dicotyledonous substrates (SH, BP, PK and DA) are higher in cellulose and lower in hemicelluloses compared with monocotyledonous cell walls (OH and BS). In any case, proportion of cellulose and lignin does not apparently affect the rate or extent of cell wall fraction of these substrates. In fact, correlation of both OMd and 48 h gas production with cellulose, considered as the difference between ADF and lignin, or lignin/cutin contents of incubated cell wall substrates rendered low coefficients (-0.015 and -0.012 for cellulose and -0.325 and -0.433 for Lignin (sa), respectively). However, correlations with hemicelluloses, considered as the difference between NDF and ADF, were higher (-0.664 for OMd and -0.685 with 48 h gas production). Miron *et al.* (2001) recorded different *in vitro* microbial degradability of cell wall polysaccharides depending on the substrate. Although the potential effect of the lignin proportion is associated with cell wall fermentation pattern (Chesson *et al.*, 1983; Thompson *et al.*, 2000), this is not necessarily a direct relationship (Jung & Allen, 1995), as the type of lignin and the extent of linkages between lignin and heteroxylan side-chains, which difficult hemicelluloses digestion, depend on the nature of the substrate (Jung & Deetz, 1993; Miron *et al.*, 2001).

Methane production can be an index of fermentation efficiency in ruminants, since up to 12% of energy intake is lost as methane (Johnson & Johnson, 1995). Compared to forages, fermentation of some non-forage fibre sources may alter rumen microbiota towards a more amylolytic population, thus leading to a higher propionate production. Propionate may act as hydrogen sink reducing the utilisation of hydrogen in methanogenesis (Wang *et al.*, 2018). Further, the smaller particle size of these feeds promotes a faster passage rate, which leads to a lower methane production (Okine *et al.*, 1989; Beauchemin *et al.*, 2008). This effect is further enhanced as highly digestible fibre sources stay in the rumen for a shorter time, and this may also restrict the time available for fermentation, leading to a lower methane production than less digestible fibre sources. Pardo *et al.* (2016) estimated a reduction of methane emission from fermentation in dairy goats when increasing proportion of agroindustrial by-products in diet. In general, total methane production (Fig. 3) agreed with gas production pattern (Fig. 1); however, among the highly fermentable sources, from 24 h onwards methane volume from BP was lower than SH, in response to its numerically lower methane proportion in total gas ($p>0.05$). The lack of a specific methane pattern, different to that

of total gas production, is expectable considering that no differences were recorded in acetate proportion, and substrates differences in that of butyrate were manifested in lower values for BP and DA, that otherwise rendered a higher VFA production at 12 h. Therefore, qualitatively, fermentation of cell wall fractions from fibrous feeds does not allow for substantial differences in methane production, apart to those expected from the quantitative extent of fermentation, that are manifested in total gas and total VFA production.

However, substantial differences were observed in propionate proportion, which was higher in BP than in PK, OH and DA. It is worth considering that propionate proportion is inversely related with methane (Moss *et al.*, 2000). Having into account that, stoichiometrically, propionate contribution to the volume of gas produced is lower than that from acetate and butyrate (Beuvink & Spoelstra, 1992; Getachew *et al.*, 1997), certain level of underestimation of cell wall fermentation from sugarbeet pulp can be assumed if measured from the total gas production, as it is reflected by its higher total VFA production. However, it was not the case with OMd, which did not differ between BP and SH. Reasons explaining differences in OM that did not match to those in gas production or VFA concentration are not apparent but respond to previously observed non-extracted substrates comparison (Ortolani *et al.*, 2020).

In summary, fermentation of the cell wall fraction of these fibrous feeds is not directly linked to its chemical composition, at least to their cellulose or lignin proportions, although a correlation with their hemicellulose content has been observed. Cell wall of sugarbeet pulp is highly and rapidly fermentable, producing a high proportion of propionate and rendering a low proportion of methane, and that from soybean hulls behaves similarly. The fermentation rate of palm kernel cake was also high during the first 24 h, so a potential contribution can be assumed if included as ingredient in concentrate compound feeds, considering the low rumen retention time of high concentrate diets. Despite structural and chemical differences of cell wall fractions from dehydrated alfalfa and barley straw, their fermentation was very similar in rate and extent.

References

Amanzougarene Z, Fondevila M, 2018. Fitting of pH conditions for the study of concentrate feeds fermentation by the *in vitro* gas production technique. Anim Prod Sci 58: 1751-1757. https://doi.org/10.1071/AN16097

Analytical Software, 2010. Statistix 10 for Windows. Analytical Software, Tallahasee, FL, USA.

AOAC, 2005. Official methods of analysis, 18th ed., Horwitz W, Latimer GW (eds.), Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Armentano L, Pereira M, 1997. Measuring the effectiveness of fiber by animal response trials. J Dairy Sci 80: 1416-1425. https://doi.org/10.3168/jds.S0022-0302(97)76071-5

Bampidis VA, Robinson PH, 2006. Citrus by-products as ruminant feeds: A review. Anim Feed Sci Technol 128: 175-217. https://doi.org/10.1016/j.anifeedsci.2005.12.002

Barrios Urdaneta A, Fondevila M, Balcells J, Dapoza C, Castrillo C, 2000. *In vitro* microbial digestion of straw cell wall polysaccharides in response to supplementation with different sources of carbohydrtes. Austr J Agric Res 51: 393-400. https://doi.org/10.1071/AR99079

Beauchemin KA, Kreuzer M, O'Mara F, McAllister TA, 2008. Nutritional management for enteric methane abatement: a review. Austr J Exp Agric 48: 21-27. https://doi.org/10.1071/EA07199

Beauchemin KA, McGinn SM, Benchaar C, Holtshausen L, 2009. Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation, and milk production. J Dairy Sci 92: 2118-2127. https://doi.org/10.3168/jds.2008-1903

Beuvink JM, Spoelstra SF, 1992. Interactions between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms *in vitro*. Appl Microbiol Technol 37: 505-509. https://doi.org/10.1007/BF00180978

BOE, 2013. Real Decreto 53/2013, de 1 de febrero, por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia. Boletín Oficial del Estado No. 34, 08/02/13.

Bradford BJ, Mullins CR, 2012. Invited review: strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. J Dairy Sci 95: 4735-4746. https://doi.org/10.3168/jds.2012-5393

Chesson A, 1993. Mechanistic model of forage cell wall degradation. In: Forage cell wall structure and digestibility; Jung HG, Buxton DR, Hatfield DR, Ralph J (eds); American Society of Agronomy Inc., Madison, WI, USA, pp: 347-376. https://doi.org/10.2134/1993.foragecellwall.c14

Chesson A, Gordon AH, Lomax JA, 1983. Substituent groups linked by alkali-labile bonds to arabinose and xylose residues of legume, grass and cereal straw cell walls and their fate during digestion by rumen microorganisms. J Sci Food Agric 34: 1330-1340. https://doi.org/10.1002/jsfa.2740341204

Cone JW, van Gelder AH, 1999. Influence of protein fermentation on gas production profiles. Anim Feed Sci Technol 76: 251-264. https://doi.org/10.1016/S0377-8401(98)00222-3

DePeters EJ, Fadel JG, Arosemena A, 1997. Digestion kinetics of neutral detergent fiber and chemical composition within some selected by-product feedstuffs. Anim Feed Sci Technol 67: 127-140. https://doi.org/10.1016/0377-8401(96)01145-5

Doreau M, Ferlay A, 1995. Effect of dietary lipids on nitrogen metabolism in the rumen: A review. Livest Prod Sci 43: 97-110. https://doi.org/10.1016/0301-6226(95)00041-I

Ertl P, Zebeli Q, Zollitsch W, Knaus W, 2015. Feeding of by-products completely replaced cereals and pulses in dairy cows and enhanced edible feed conversion ratio. J Dairy Sci 98: 1225-1233. https://doi.org/10.3168/jds.2014-8810

EU, 2010. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. 22 Sept 2013 [CELEX:32010L0063].

FEDNA, 2019. Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos, 4th ed.; de Blas C, *et al.* (eds.). Fundación Española para el Desarrollo de la Nutrición Animal, Madrid.

Ford CW, Elliot R, 1989. Biodegradability of grass cell walls in relation to chemical composition and rumen microbial activity. J Agr Sci, Camb, 108: 201-209. https://doi.org/10.1017/S0021859600064273

Garleb KA, Bourquin LD, Hsu JT, Wagner GW, Schmidt SJ, Fahey Jr GC, 1991. Isolation and chemical analysis of nonfermented fiber fractions of oat hulls and cottonseed hulls. J Anim Sci 69: 1255-1271. https://doi.org/10.2527/1991.6931255x

Gasa J, Castrillo C, Baucells MD, Guada JA, 1989. By-products from the canning industry as feedstuff for ruminants: Digestibility and its prediction from chemical composition and laboratory bioassays. Anim Feed Sci Technol 25: 67-77. https://doi.org/10.1016/0377-8401(89)90108-9

Getachew G, Blümmel M, Makkar HPS, Becker K, 1997. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim Feed Sci Technol 72: 261-281. https://doi.org/10.1016/S0377-8401(97)00189-2

Getachew G, Robinson PH, dePeters EJ, Taylor SJ, 2004. Relationship between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. Anim Feed Sci Technol 111: 57-71. https://doi.org/10.1016/S0377-8401(03)00217-7

González Ronquillo M, Fondevila M, Barrios Urdaneta A, Newman Y, 1998. *In vitro* gas production from buffel grass (Cenchrus ciliaris L.) fermentation in relation to the cutting interval, the level of nitrogen fertilisation and the season of growth. Anim Feed Sci Technol 72: 19-32. https://doi.org/10.1016/S0377-8401(97)00181-8

Grant RJ, 1997. Interactions among forages and nonforage fiber sources. J Dairy Sci 80: 1438-1446. https://doi.org/10.3168/jds.S0022-0302(97)76073-9

Hindle V, Steg A, van Vuuren AM, Vroons-de Bruin J, 1995. Rumen degradation and post-ruminal digestion of palm-kernel by products in dairy cows. Anim Feed Sci Technol 51: 103-121. https://doi.org/10.1016/0377-8401(94)00677-2

Hsu JT, Faulkner DB, Garleb KA, Barclay GC, Fahey Jr GC, Berger LL, 1987. Evaluation of corn fiber, cottonseed hulls, oat hulls and soybean hulls as roughage sources for ruminants. J Anim Sci 65: 244-255. https://doi.org/10.2527/jas1987.651244x

Johnson KA, Johnson DE, 1995. Methane emissions from cattle. J Anim Sci 73: 2483-92. https://doi.org/10.2527/1995.7382483x

Jung HG, Deetz DA, 1993. Cell wall lignification and degradability. In: Forage cell wall structure and digestibility; Jung HG; Buxton DR, Hatfield RD, Ralph J (eds). p 315. ASA-CSSA-SSSA, Madison, WI, USA. https://doi.org/10.2134/1993.foragecellwall

Jung HG, Allen MS, 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J Anim Sci 73: 2774-2790. https://doi.org/10.2527/1995.7392774x

Krause DO, Denman SE, Mackie RI, Morrison M, Rae AL, Attwood GT, McSweeney GS, 2003. Opportunities to improve fiber degradation in the rumen: microbiology, ecology and genomics. FEMS Microbiol Rev 27: 663-693. https://doi.org/10.1016/S0168-6445(03)00072-X

Mertens DR, 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J AOAC Int 85: 1217-1240.

Miron J, Yosef E, Ben-Ghedalia D, 2001. Composition and *in vitro* digestibility of monosaccharide constituents of selected byproduct feeds. J Agric Food Chem 49: 2322-2326. https://doi.org/10.1021/jf0008700

Moss A, Jouany JP, Newbold J, 2000. Methane production by ruminants: its contribution to global warming. Ann Zootech 49: 231-253. https://doi.org/10.1051/animres:2000119

Mould FL, Kliem KE, Morgan R, Mauricio RM, 2005. *In vitro* microbial inoculum: A review of its function and properties. Anim Feed Sci Technol 123-124: 31-50. https://doi.org/10.1016/j.anifeedsci.2005.04.028

Münnich M, Khiaosa-ard R, Klevenhusen F, Hilpold A, Khol-Parisini A, Zebeli Q, 2017. A meta-analysis of feeding sugar beet pulp in dairy cows: Effects

on feed intake, ruminal fermentation, performance, and net food production. Anim Feed Sci Technol 224: 78-89. https://doi.org/10.1016/j.anifeedsci.2016.12.015

Okine EK, Mathison GW, Hardin RT, 1989. Effects of changes in frequency of reticular contractions on fluid and particulate passage rates in cattle. J Anim Sci 67: 3388-3396. https://doi.org/10.2527/jas1989.67123388x

Ortolani IR, Amanzougarene Z, Fondevila M, 2020. *In vitro* estimation of the effect of grinding on rumen fermentation of fibrous feeds. Animals 10: 732. https://doi.org/10.3390/ani10040732

Pardo G, Martín-García I, Arco A, Yáñez-Ruiz DR, Moral R, del Prado A, 2016. Greenhouse-gas mitigation potential of agro-industrial by-products in the diet of dairy goats in Spain: a life-cycle perspective. Anim Prod Sci 56: 646-654. https://doi.org/10.1071/AN15620

Pereira MN, Garrett EF, Oetzel GR, Armentano LE, 1999. Partial replacement of forage with nonforage fiber sources in lactating cow diets. I. Performance and health. J Dairy Sci 82: 2716-2730. https://doi.org/10.3168/jds.S0022-0302(99)75528-1

Robertson JB, Van Soest PJ, 1981. The detergent system of analysis and its application to human foods. In: The analysis of dietary fiber in foods; James WPT, Theander O (eds.), Marcel Dekker, NY, pp: 123-158.

Seo S, Lee SC, Lee SY, Seo JG, Ha JK, 2009. Degradation kinetics of carbohydrate fractions of feeds using automated gas production technique. Asian Austral J Anim Sci 22: 356-364. https://doi.org/10.5713/ajas.2009.80613

Smith LW, Waldo DR, 1969. Method for sizing forage cell wall particles. J Dairy Sci 52: 2051-2053. https://doi.org/10.3168/jds.S0022-0302(69)86898-0

Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J, 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48: 185-197. https://doi.org/10.1016/0377-8401(94)90171-6

Thompson RK, Mustafa AF, Mckinnon JJ, Maenz D, Rossnagel B, 2000. Genotypic differences in chemical composition and ruminal degradability of oat hulls. Can J Anim Sci 80: 377-379. https://doi.org/10.4141/A99-132

Van Soest PJ, 1994. Nutritional ecology of the ruminant, 2nd ed. Cornell Univ Press, Ithaca, NY. https://doi.org/10.7591/9781501732355

Wang K, Nan X, Chu K, Tong J, Yang L, Zheng S, Zhao G, Jiang L, Xiong B, 2018. Shifts of hydrogen metabolism from methanogenesis to propionate production in response to replacement of forage fiber with non-forage fiber sources in diets *in vitro*. Front Microbiol 9: 2764. https://doi.org/10.3389/fmicb.2018.02764

Wilson JR, 1994. Cell wall characteristics in relation to forage digestion by ruminants. J Agric Sci, Camb 122: 173-182. https://doi.org/10.1017/S0021859600087347

Zhang Y, Gao W, Meng Q, 2007. Fermentation of plant cell walls by ruminal bacteria, protozoa and fungi and their interaction with fibre particle size. Arch Anim Nutr 61: https://doi.org/10.1080/17450390701204020