In vitro acidification potential and fermentation pattern of cereal grains incubated with inoculum of animals given forage or concentrate based diets

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Abstract

The potential of acidification and fermentation pattern of maize (M1, M2 and M3), barley (B1, B2 and B3) and sorghum (S1, S2 and S3) varieties was studied in three 10 h *in vitro* incubation series with a low buffered medium using inoculum from rumen contents of sheep given a forage (FI) or a concentrate (CI) diet. From 2 to 10 h average pH with CI was lower than with FI (P<0.001), final pH being 5.25 and 5.76(SEM=0.013), respectively. With CI, incubation pH was lowest (P<0.001) with the three barley varieties from 2 to 6 h, and highest at 8 and 10 h with sorghum, maize recording intermediate values (average pH at 8 h of 5.63, 5.44 and 5.13, respectively). With FI, pH with barley was lowest than with maize or sorghum (average pH at 10 h of 5.52, 5.85 and 5.91, respectively). No pH differences among varieties within cereal species were detected (P>0.05). The average gas production with CI was always superior (P<0.001) to that with FI (673 *vs.* 36.8 mL/g OM at 10 h, SEM = 1.02). Concentration of volatile fatty acids (VFA) with CI was higher than FI (31.0 *vs.* 19.9 mmol/L at 8 h; P<0.001), as well as proportions of butyrate and valerate (P<0.001), whereas proportions of acetate and branched chain VFA were lower (P<0.001), and propionate did not differ among inocula. Among substrates, the volume of gas produced from barley varieties with CI was similar to that from maize (P>0.05), except at 4 and 6 h, when M2 and M1 were lower. No differences among varieties of either barley or maize were detected at any time (P>0.05), but the tannin-free S1 was grouped with maize whereas gas production with S2 and S3 was lower (P<005). After 10 h, barley and maize varieties produced on average 82.5 and

73.0 mL/g OM, whereas S1, S2 and S3 rendered 68.4, 31.1 and 39.7 mL/g OM. With FI, differences between barley and maize were detected after 6 h, resulting in average values of 61.0, 35.3 and 14.1 mL/g OM at 10 h for barley, maize and sorghum. Gas production results were supported by total VFA concentration. The fermentative activity for vitreous starch of a forage inoculum is lower than that promoted by a concentrate diet, thus affecting substrate utilization and rumen environment. Fermentation of starch-rich substrates in a low buffered medium gives a more realistic picture than conventionally buffered conditions.

Keywords: cereal grains; pH; inoculum; *in vitro* fermentation.

Abbreviations: ADF, acid detergent fibre; ADL, acid deterngent lignin; aNDFom, neutral detergent fibre; BCFA, branched chain fatty acids; CI, concentrate inoculum; DM, dry matter; EE, ether extract; FI, forage inoculum; OM, organic matter; SEM, standard error of means; VFA, volatile fatty acids.

1. Introduction

Cereal grains vary in their rate of starch rumen fermentation, which is higher in wheat, oats and barley than maize and sorghum (Herrera-Saldana et al., 1990; O´Brien, 1999). Besides, the presence of phenolic compounds in some varieties of sorghum grains markedly affects their microbial fermentation (Streeter et al., 1990; Kim et al., 2006). Nutritive values of cereal grains are often assumed as common for each vegetal species; however, notable differences reported among varieties (O´Brien, 1999) prevent for such generalisation.

The study of potential rumen microbial fermentation of cereal grains and the comparison of different species and varieties has been approached by *in situ* incubation (Nocek and Tamminga, 1991; Offner et al., 2003). Since these studies are costly, laborious and time consuming, the *in vitro* gas production technique has been alternatively applied to study the pattern of fermentation of whole grains (Opatpatanakit et al., 1994; Chai et al., 2004; Lanzas et al., 2007) or their starch or fibre components (Chen et al., 1999; Tahir et al., 2013).

However, this technique does not consider the range of rumen pH, below 6.0 and even under 5.5, resulting from the high production of volatile fatty acids (VFA) and lactate by starch fermentation, together with the low salivation promoted by concentrate diets rich in cereal grains (Krause and Oetzel, 2006; Nagaraja and Titgemeyer, 2006). The acidification capacity of cereal grains is associated with their amount of starch, as well as with its chemical nature and its availability for rumen microbes (Van Barneveld, 1999; Offner et al., 2003). Therefore, the study of microbial fermentation of starch rich feeds should require the establishment of low incubation pH, whereas the procedures for the *in vitro* gas production technique are carried out on incubation under well buffered conditions (Mould et al., 2005a). By reducing the proportion of bicarbonate ion used as buffer in the incubation solution, our group (Amanzougarene and Fondevila, in evaluation) successively adjusted the incubation pH at a range level from 6.50 to 5.75.

Different species of rumen bacteria are able to ferment starch in the rumen; further, their activity is also related to the nature of the starch source (McAllister et al., 1990; Klieve et al., 2003). In practical situations, ruminant animals not adapted to high starch diets, such as cattle reared at pasture that are introduced to intensive feeding systems in Mediterranean countries, have a reduced capacity of VFA absorption through the rumen wall that, together with a high production of lactic acid, contributes to the appearance of acidosis. This *in vitro* work aimed to study the acidification capacity and the fermentation pattern of three varieties of each barley, maize and sorghum grains by using a poorly buffered medium, and to what extent such effects depend on the characteristics of the rumen inoculum, either from a forage or a concentrate diet.

2. Material and methods

2.1. Substrates and inocula

78 Nine cereal feeds, consisting of three varieties of maize (Dekalb 6815, M1; Dekalb
79 6667YG, M2; Pioneer 0725, M3), three of sorghum (one white sorghum, S1, and two brown
80 sorghums, S2 and S3; unknown varieties) and three of barley (var. Gustav, B1; var. Signora, B2;
81 var. Graphic, B3), were incubated. All substrates were milled through a sieve of 1 mm using a
82 hammer mill (Retsch Gmbh/SK1/417449). Chemical composition of substrates is given in Table 1.
83 Four adult Rasa Aragonesa ewes (average weight 71.5 ± 1.7 kg) fitted with a rumen
84 cannula, were used as donor of rumen inoculum. Animals were housed in the facilities of the
85 Servicio de Apoyo a la Experimentación Animal of the Universidad de Zaragoza, Spain. Animal care
86 and procedures for extraction of rumen inoculum were approved by the Ethics Committee for
87 Animal Experimentation. Care and management of animals agreed with the Spanish Policy for
88 Animal Protection RD 53/2013, which complies with EU Directive 2010/63 on the protection of
89 animals used for experimental and other scientific purposes. At the beginning of the experiment,
90 ewes were fed 600 g of alfalfa hay plus 300 g of straw for two weeks, in order to get a rumen
91 environment characteristic of forage diets (forage inoculum, FI). Then, diet was changed to 500 g
92 of concentrate (0.60 barley, 0.20 corn, and 0.20 soybean meal) plus 300 g of alfalfa hay for
93 another two weeks, as a typical concentrate-fed animals (concentrate inoculum, CI). Rumen
94 contents (approximately 300 ml) were extracted before the morning feeding, pooled, filtered
95 through cheesecloth and transferred to the laboratory in thermos bottles preheated to 39° C.
96
97 2.2 *Incubation procedures*
98 The *in vitro* incubation of substrates followed the procedure of Theodorou et al. (1994), in
99 glass bottles (116 mL total volume) containing 500 mg of substrate. The bottles were filled with 80
100 mL of incubation solution, which consisted of 0.10 rumen inoculum and 0.90 of an incubation
101 mixture containing (mL/L): 474 mL distilled water; 238 mL of buffer solution made up with 1.9 g/L
102 sodium bicarbonate ($NaHCO_3$) and 0.1 g/L ammonium bicarbonate ($(NH_4)HCO_3$), calculated to
103 establish a minimum pH of 5.50 (Kohn and Dunlap, 1998) and assumed as an almost pH-free

104 medium (Amanzougarene and Fondevila, in evaluation); 238 mL of macro-minerals solution made
105 up with 5.7 g disodium hydrogen phosphate (Na_2HPO_4), 6.2 g potassium di-hydrogen
106 orthophosphate (KH_2PO_4), and 0.6 g magnesium sulphate ($MgSO_4.7H_2O$); and 50 mL of reducing
107 solution made up with 47.5 mL distilled water, 2 mL 1N NaOH and 313 mg HCl-cysteine. Micro-
108 minerals solution and resazurin were not included in the incubation medium (Mould et al.,
109 2005a). Ingredients were mixed and allowed to be reduced under a CO_2 atmosphere. Bottles were
110 filled with the incubation solution under a CO_2 stream, sealed with rubber caps and aluminium
111 rings, and incubated at 39 ºC. Three 10 h runs of incubation with five bottles per treatment were
112 carried out with each type of inoculum (FI and CI). On each incubation run, three additional
113 bottles without substrate were also included as blanks, for subtracting the contribution of
114 inoculum to overall gas production. Pressure was recorded every two hours of incubation, by
115 means of an HD 2124.02 manometer fitted with a TP804 pressure gauge (Delta Ohm, Caselle di
116 Selvazzano, Italy). Pressure readings were converted into volume by a pre-established linear
117 regression equation between the pressure recorded in the same bottles under the same
118 conditions and known air volumes (n= 103; R^2= 0.996). The gas volume recorded for each
119 incubation time was expressed per unit of incubated organic matter (OM). One bottle per
120 treatment was opened every 2 h and at the end of each incubation run, for measuring incubation
121 pH (CRISON micropH 2001, Barcelona, España). Incubation medium in the bottle opened after 4
122 and 8 h was sampled and stored at -20ºC for volatile fatty acids (VFA) analysis (2 mL sample,
123 collected over 0.5 mL of a deproteinizing mixture of 0.5M PO_4H_3 with 2 mg/mL 4-methyl valeric
124 acid) and lactate (2 mL).
125
126 *2.3. Chemical analyses*
127 Substrates were analysed following the procedures of AOAC (2005) for dry matter (DM; ref.
128 934.01), organic matter (OM; ref. 942.05), crude protein (CP; ref. 976.05) and ether extract (EE;
129 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, New York), using α–amylase and sodium sulphite, and results are expressed exclusive of residual ashes. The acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined as described by AOAC (2005) and Robertson and 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). The total phenolic (TP) and tannin (TT) content was analysed following the colorimetric method of Makkar et al. (1993) using the Folin-Ciocalteau reagent and with tannic acid (MERCK Chemicals, Madrid, Spain) as the reference standard. The frozen samples of incubation media were thawed and centrifuged at 20,000 g for 15 minutes for their analysis of lactate 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 x 530 µm id). The lactate concentration was determined by the colorimetric method proposed by Barker and Summerson (1941).

2.4. Statistical analysis

Results were analysed by ANOVA using the Statistix 10 software package (Analytical Software, 2010). The effect of the type of inoculum, the cereal sources and their interaction on the gas production, pH, total VFA concentration, VFA profile and lactate were studied as factors for each time of sampling, considering the interaction inoculum x incubation run as block. The differences were considered significant when $P<0.05$, and a trend for significance was considered when $0.05 \leq P<0.10$. The Tukey test ($P<0.05$) was used for the multiple comparison between means. The relationship among results from the different parameters was studied by linear or multiple regression analyses, at a level of significance of 0.05.

3. Results

155 At the start of the incubation series, the mean inoculum pH was 6.35 ± 0.16 and 6.73 ±
156 0.19 for the concentrate (CI) and forage (FI) inocula, respectively (n=3), and dropped to average
157 values of 5.82 and 5.99, respectively, in the first two hours of incubation (SEM=0.005). During the
158 whole incubation period, average pH with CI was lower than that of FI (P<0.001), differences
159 increasing up to 0.51 pH units at 10 h incubation (pH of 5.25 and 5.76 for CI and FI; SEM=0.013).
160 Since the interaction inoculum x substrate resulted significant at 2, 8 and 10 h of incubation
161 (P<0.01), the pH pattern followed with each substrate is presented separately for each inoculum.
162 Incubation pH from 2 to 6 h incubation with CI (Figure 1a) was lowest (P<0.001) with the three
163 varieties of barley, but differences among sorghum and maize were not manifested until 8 and 10
164 h, when pH was highest with sorghum and recording intermediate and minimum values with
165 maize and barley varieties (average pH at 8 h of 5.63, 5.44 and 5.13 for sorghum, maize and
166 barley, respectively). No differences in pH among varieties of each cereal species were detected
167 with CI (P>0.05), except at the end of incubation (10 h), when pH for sorghum S1 was lower than
168 for S2, and that of M3 lower than for M1 (5.42 *vs.* 5.61 and 5.16 vs. 5.32, respectively; P<0.05),
169 whereas the pH recorded at 10 h with M1 and S1 did not differ (P>0.05). When cereals were
170 incubated using FI (Figure 1b), the pH from 2 to 10 h incubation was reduced at a lower extent
171 than with CI. At 8 and 10 h incubation, pH with barley varieties (on average, 5.68 and 5.52,
172 respectively) was lowest than with maize (5.93 and 5.85 at 8 and 10 h) or sorghum (5.96 and
173 5.91). No differences in incubation pH were detected among varieties within cereals, nor between
174 sorghum and corn varieties except with M1 at 6 and 8 h incubation (P>0.05).
175 Between inocula, once corrected for the gas production in blank bottles the average gas
176 production recorded with CI was always superior (P<0.001) to the gas production recorded with FI
177 (Figure 2), with differences increasing with time from 8 to 31 mL/g OM (average gas production of
178 67.3 and 36.8 mL/g OM for CI and FI at 10 h incubation; SEM = 1.02. The pattern of gas production
179 among cereal varieties of each cereal species differed for each inoculum (P= 0.001), and for an
180 easier understanding results are reported separately for CI and FI. When CI was used (Figure 3a),

181 the volume of gas produced from barley varieties was similar to that from maize (P>0.05), except
182 at 4 and 6 h incubation, when M2 and M1 gave lower values than the barleys. No differences
183 among varieties of either barley or maize were detected at any incubation time (P>0.05). In
184 contrast, sorghum varieties showed a different behaviour (P<005): whereas S1 was grouped with
185 maize varieties, S2 and S3 produced a lower amount of gas. As a result, after 10 h incubation,
186 barley and maize varieties produced on average 82.5 and 73.0 mL/g OM, whereas S1, S2 and S3
187 rendered 68.4, 31.1 and 39.7 mL/g OM), respectively. However, with FI (Figure 3b) no differences
188 between barley and maize substrates were detected up to 6 h, and afterwards substrates ranked
189 as: B1, B3, B2 > M1, M2 > M3, S1, S3 > S2 (P<0.05), resulting in average values of 61.0, 38.9, 22.9
190 and 1.5 mL/g OM at 10 h for each mentioned group.
191 At both sampling times CI showed a higher (P<0.001) concentration of VFA (22.8 *vs.* 14.8
192 mmol/L at 4 h and 31.0 *vs.* 19.9 mmol/L at 8 h) than FI, as well as higher (P<0.001) proportions of
193 butyrate (0.107 *vs.* 0.052 and 0.105 *vs.* 0.053) and valerate (0.009 *vs.* 0.006 at both 4 and 8 h) and
194 lower (P<0.001) proportions of acetate (0.479 *vs.* 0.542 and 0.469 *vs.* 0.531) and branched chain
195 VFA (sum of isobutyrate and isovalerate, BCFA; 0.013 *vs.* 0.020 and 0.012 *vs.* 0.019), whereas
196 propionate did not differ among inocula (average proportion of 0.177 and 0.191 at 4 and 8 h).
197 Since the inoculum x substrate interaction was significant only for butyrate proportion at
198 4 h (P= 0.009) and for acetate and butyrate proportions at 8 h (P<0.001), only substrate means for
199 total VFA and molar VFA are shown in Tables 2 and 3, and the implications of such interactions
200 are explained in text. On average, the VFA concentration at 4 h (Table 2) was higher (P= 0.001)
201 with the barley variety B3 than all maize and sorghum varieties, and propionate proportion was
202 higher (P<0.001) for barley than sorghum varieties, with maize showing intermediate values. In
203 contrast, acetate proportion was higher (P<0.001) in sorghum than barley varieties. Concerning
204 butyrate proportion at 4 h (P= 0.009), S2 showed a higher proportion than B1, B2, M1 and M2
205 when incubated with CI, but no substrate differences were recorded with FI. The BCFA proportion
206 after 4h of incubation was higher (P= 0.004) in S3 than in B2, B3, M2 and M3, whereas no

207 differences were recorded in valerate proportion. No differences were detected among varieties
208 of the same cereal species for total VFA concentration or any VFA molar proportion.

209 After 8 h, a higher (*P*>0.001) VFA concentration was detected with barley varieties
210 compared with S2 and S3, and B2 was also higher than S1, whereas those of maize recorded
211 intermediate values (Table 3). As for 4h, no differences were detected among varieties of the
212 same cereal species. Propionate proportion in barley varieties and M1 was higher (*P*<0.001) than
213 in sorghums, but M2 and M3 did not differ from S3. With FI as inoculum, fermentation of
214 sorghum varieties rendered a higher proportion of acetate than the three barleys, those of maize
215 recording intermediate values, whereas no substrate differences were detected with CI.
216 Concerning butyrate, its proportion was lowest for barley when incubated with CI, but it did not
217 differ among substrates with FI.

218 No differences among inocula were detected in lactic acid concentration at 4 h but it was
219 higher (*P*=0.001) with CI than FI at 8 h incubation (2.31 *vs.* 1.33 mM; SEM=0.225). Among
220 substrates, lactate concentration at 4 h was higher in barley varieties than in those of sorghum
221 and M2 and M3 (P<0.001, data not shown), whereas after 8 h lactate concentration in barley
222 varieties were highest (average values of 3.92, 3.94, 3.97, 0.75, 0.96, 1.17, 0.35, 0.26 and 0.26 mM
223 for B1, B2, B3, M1, M2, M3, S1, S2 and S3; SEM= 0.478; P<0.001).

224

225 **4. Discussion**

226 When ruminants at pasture are introduced to intensive systems based on cereal-rich diets
227 there is an acute drop of rumen pH associated to the higher extent of fermentation (Krause and
228 Oetzel, 2006; Nagaraja and Titgemeyer, 2006). Our aim in the present work was to determine the
229 magnitude of fermentation differences between concentrate- and forage-type environments,
230 considering the potential application of results to practical situations of feeding transition. In such
231 scenario, it is worth knowing the potential of acidification of the different substrates, since this

may determine either an easier or a more difficult adaptation of the rumen, thus minimising at possible the risk of acidosis (Broudiscou et al., 2014).

The amount of starch of the tested varieties of cereal species agrees with that reported by FEDNA (2010), being higher in both sorghum and corn than in barley. Rumen microbial fermentation of different cereal species and varieties within a certain species has been previously compared at a buffered pH (O´Brien, 1999; Chai et al., 2004; Lanzas et al., 2007). Since other factors such as the geographical site or the year of growth also affect the result (Opatpanatakit et al., 1994; O´Brien, 1999), this work was rather focused to the different potential of acidification of the rumen environment and its effect on microbial utilisation, in order to estimate which can be better suited to a gradual adaptation of a fibrolytic to an amylolytic microbiota. Most *in vitro* approaches to this had been limited because studies were mostly carried out on a well-buffered medium, and therefore results can hardly be extrapolated to practical situations. However, at the same time results from this experiment are difficult to compare with others in literature, since approaches to gas production allowing for a relatively free incubation pH are scarce (Opatpanatakit et al., 1994). In incubation conditions with buffered medium, Chai et al. (2004) observed that gas production from maize after 6 h was 0.36 that of barley, whereas such proportion increased to 1.06 after 12 h incubation. Lanzas et al. (2007) observed higher fractional rate of gas production with barley than maize and sorghum varieties (on average, 0.24, 0.15 and 0.06 h^{-1}), showing interspecies ranges between 0.22 and 0.29, 0.09 and 0.17, and 0.05 and 0.08, respectively. Opatpanatakit et al. (1994) used the McDougall (1948) artificial saliva, which allows for a more acute drop of pH. They also observed highest gas production with barley, intermediate with maize and lowest with sorghum (on average, 222, 138 and 104 mL/g DM, respectively), under pH values after 7 h incubation ranging from 5.7 to 6.1 for barley, 6.5 to 6.9 for maize and 6.5 to 6.8 for sorghum.

The effect of the source of rumen fluid on the *in vitro* fermentation has been recently demonstrated by Broudiscou et al. (2014), and it is widely accepted that forage based diets

maintain higher incubation medium pH than diets including a major proportion of concentrate. Despite the magnitude of the drop of pH caused by fermentation was more pronounced, the incubation environment promoted by giving a concentrate diet to the donor animals was more favourable for cereal fermentation than that induced by a fibrous diet, as expected (Menke and Steingass, 1988; Mould et al., 2005b). Throughout the 10 h incubation period, pH was more stable with the forage inoculum, probably because of its lower capability to ferment starch and the lower extent of fermentation caused by the lack of adaptation of microbial communities to starch substrates (Trei et al., 1970; Nagadi et al., 2000). In any case, pH in maize and sorghum incubations with FI was around 6.0, considered as a threshold for viability of most non-amylolytic rumen bacterial species (Russell and Dombrowski, 1980), and even reached to 5.5 after 10 h incubation with barley. With inoculum from a concentrate diet, pH dropped at a higher extent as the incubation proceeded and reached lower values with all substrates, allowing for clearer differences among substrates. This was also manifested in a higher VFA concentration, as noted by Calsamiglia et al. (2008), but also in butyrate proportion at the expense of that of acetate and BCFA.

The accumulated volume of gas recorded from the blanks of inocula (without substrate) after 10 h incubation with FI and CI were 20.7 and 31.0 mL (from 80 mL, data not shown), produced by fermentation of solubles suspended in the medium. Once this amount was subtracted from the total recorded volume, the volume of gas produced from substrates was also higher with CI than FI as inoculum, in 1.3 to 1.4-fold for the three barleys, from 1.8 to 2.0-fold for M1 and M2, and from 2.3 to 3.0 for M3, S1 and S3. It has to be considered that, despite the source of inoculum, the pH along all the 10 h incubation was below 6.0, being lowest with barley varieties, with which ranged from 5.9 to 5.5 with FI and from 5.7 to 5.0 with CI, seriously challenging microbial activity at the last part of incubation. In fact, the extent of fermentation in terms of gas production was lower than what has been reached in well-buffered media from cereal grains (Chai et al., 2004; Lanzas et al., 2007; Tahir et al., 2013). *In vivo,* Offner and Sauvant

(2004) established a linear relationship between pH in the range from 6.4 to 5.7 and rumen digestion of starch; however, Shriver et al. (1986) did not observe differences in *in vitro* digestibility of non-structural carbohydrates of a concentrate feed between incubation pH of 5.8 and 7.0. Further, the contribution of fermentation acids produced to the volume of gas is lower at low pH, because of the reduced contribution of the indirect gas from the buffer (Amanzougarene and Fondevila, in evaluation), and therefore the magnitude of differences among inocula are expected to be higher in the case of barleys.

Compared with corn and sorghum, it is expected that the starch of barley was degraded more rapidly (Opatpanatakit et al., 1994; Lanzas et al., 2007). Whereas it is apparent that barley produced more gas and at a faster rate than maize and sorghum with FI, differences between barley and corn were lower when the inoculum came from a highly concentrate diet. In this sense, it is worth noting that fermentation of both corn and sorghum varieties is clearly low when FI was used, suggesting that the microbial population must be adapted to the more recalcitrant type of starch in these cereals. The different structure of starch among cereal species, or even among varieties (Van Barneveld, 1999), concretely the structure of the endosperm and the proportion of amylose (Huntington, 1997; Offner et al., 2003) may affect the rate and extent of starch fermentation, thus explaining differences in the volume of gas produced and VFA concentration between substrates. Besides, the protein matrix in maize and sorghum limits the access of rumen microbes to starch granules (McAllister et al., 1993; Rooney and Pflugfelder, 1986). The white sorghum S1, with a low phenolic and tannin content, reached similar values than maize varieties when incubated with inoculum from a concentrate diet. However, with FI, the gas production from maize varieties, mainly M3, as well as that from S1, were reduced at a higher extent than those of barleys, indicating that such inoculum is less capable to ferment the vitreous starch and the protein matrix in the endosperm of the former cereals (Philippeau and Michalet-Doreau, 1997; Ramos et al., 2009). Further, differences among varieties were apparent for sorghum, probably due to their different contents of phenolics and tannins (Table 1). With CI, gas

production from S1 was higher than that from the two brown varieties S3 and S2, because of the lower content of tannins and other phenolics in the former (Hibberd et al., 1985; Streeter et al., 1990; Opatpatanakit et al., 1994), but with FI differences between S1 and S3 were minimised. In the case of S2, the gas production was almost nil when incubated with FI.

The higher total VFA and lactate production with barley varieties are the cause of their higher acidification capacity, as it has been previously reported by Russell and Hino (1985) and Broudiscou et al. (2014). In fact, a strong correlation was observed in this work between incubation pH and lactate concentration at 8 h incubation (R^2 = 0.98). Differences in the molar proportion of VFA indicate that the fermentation profile of cereal grains differ according to the type and availability of starch, as well as with the phenolic and tannin content of sorghum varieties, in agreement with the gas production pattern.

5. Conclusions

Among the studied cereal species, the extent and rate of fermentation of barley grains were higher than that of maize varieties, contributing to the magnitude of the drop of medium pH in the first hours of incubation, and there were no major variation among the studied varieties of each species. However, despite of the minor differences among varieties of barley and maize, care must be taken in generalising experimental results to a species level. The response of sorghum varieties is largely dependent of their content in tannins and phenolic compounds, and whereas sorghum S1 behaved similarly to the maize substrates, fermentation of brown sorghums S2 and S3 depended on their amount of these secondary compounds. Fermentation of barley and maize grains is increased when inoculum from a concentrate-based diet is used, which contributes to a faster drop of pH that a forage inoculum. However, the extent of differences is higher among maize varieties, supporting that microbial community of a forage inoculum is less able to ferment vitreous types of starch. Despite the origin of inoculum, incubation pH was more stable with

sorghum substrates, but gas production from S2, with a higher proportion of phenolic compounds was almost nil with forage inoculum, indicating a higher sensibility of this microbial community.

In any case, despite it seems clear that a rapid establishment of an acidic rumen environment is useful to maximise the rumen utilisation of cereals in ruminants given high concentrate diets, the impact of such situation in overall health status of the animals and consequently their productive performances were not evaluated with this experiment, and will deserve further consideration.

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Table 1. Chemical composition (g/kg DM) of incubated substrates (varieties of maize, sorghum and barley).

Code[1]	OM[2]	CP	EE	Starch	aNDFom	ADF	ADL	TP	TT
M1	987	85	38	693	88	28	5.2		
M2	986	75	34	706	91	25	2.0		
M3	986	73	41	707	93	27	5.2		
S1	984	109	40	689	98	47	14.4	1.5	0.2
S2	985	106	41	638	93	60	16.7	19.3	8.1
S3	979	113	11	647	97	60	5.2	2.6	1.3
B1	978	105	24	672	173	56	17.5		
B2	978	112	18	635	152	44	9.3		
B3	977	91	18	651	189	55	9.1		

[1]: M1, M2 and M3 are varieties of maize; S1, S2 and S3 are varieties of sorghum; B1, B2 and B3 are varieties of barley

[2]: OM: organic matter; CP: crude protein; EE: ether extract; aNDFom: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; TP: total phenolics; TT: total tannins

451 **Table 2.** Average total volatile fatty acids concentration (VFA, mM) and molar VFA proportions
452 (mmol/mmol) recorded at 4 h incubation of varieties of maize, sorghum and barley in a low
453 buffered medium.
454

Substrate[1]	VFA	Acetate	Propionate	Butyrate	BCFA[2]	Valerate
M1	17.0^{bc}	0.513^{abc}	0.179^{abc}	0.079^{ab}	0.016^{ab}	0.007
M2	17.6^{bc}	0.514^{ab}	0.178^{bc}	0.079^{ab}	0.016^{b}	0.007
M3	17.8^{bc}	0.510^{abc}	0.180^{ab}	0.080^{ab}	0.015^{b}	0.007
S1	17.8^{bc}	0.520^{a}	0.164^{cd}	0.082^{ab}	0.018^{ab}	0.008
S2	16.3^{c}	0.526^{a}	0.159^{d}	0.083^{a}	0.019^{ab}	0.009
S3	17.5^{bc}	0.519^{a}	0.161^{d}	0.082^{ab}	0.021^{a}	0.010
B1	20.8^{abc}	0.499^{bc}	0.191^{ab}	0.079^{ab}	0.016^{ab}	0.008
B2	21.2^{ab}	0.501^{bc}	0.191^{ab}	0.078^{b}	0.016^{b}	0.008
B3	23.0^{a}	0.496^{c}	0.195^{a}	0.080^{ab}	0.015^{b}	0.008
SEM[3]	1.02	0.0036	0.0035	0.0010	0.0010	0.0007

455 [a, b, c] Different superscripts within a column indicate means differences (P<0.05)

456 [1]: M1, M2 and M3 are varieties of maize; S1, S2 and S3 are varieties of sorghum; B1, B2 and B3 are
457 varieties of barley

458 [2]: BCFA: branched-chain fatty acids (sum of isobutyrate + isovalerate)

459 [3]: SEM: standard error of the means

460 **Table 3.** Average total volatile fatty acids concentration (VFA, mM) and molar VFA proportions
461 (mmol/mmol) recorded at 8 h incubation of varieties of maize, sorghum and barley in a low
462 buffered medium.
463

Substrate[1]	VFA	Acetate	Propionate	Butyrate	BCFA[2]	Valerate
M1	24.2^{bc}	0.495^{cde}	0.196^{ab}	0.082^{abc}	0.014	0.007
M2	26.0^{abc}	0.504^{bcd}	0.188^{bcd}	0.080^{abc}	0.014	0.007
M3	25.2^{abc}	0.499^{bcde}	0.193^{abc}	0.081^{abc}	0.014	0.007
S1	23.8^{bc}	0.510^{ab}	0.177^{de}	0.084^{a}	0.015	0.007
S2	21.6^{c}	0.522^{a}	0.164^{e}	0.081^{abc}	0.018	0.009
S3	21.5^{c}	0.506^{bc}	0.178^{cde}	0.083^{ab}	0.018	0.008
B1	28.3^{ab}	0.487^{e}	0.207^{a}	0.077^{c}	0.015	0.008
B2	30.5^{a}	0.486^{e}	0.207^{a}	0.078^{bc}	0.015	0.008
B3	27.4^{ab}	0.492^{de}	0.204^{ab}	0.077^{c}	0.014	0.008
SEM[3]	1.18	0.0030	0.0035	0.0012	0.0009	0.0005

464 [a, b, c] Different superscripts within a column indicate means differences (P<0.05)

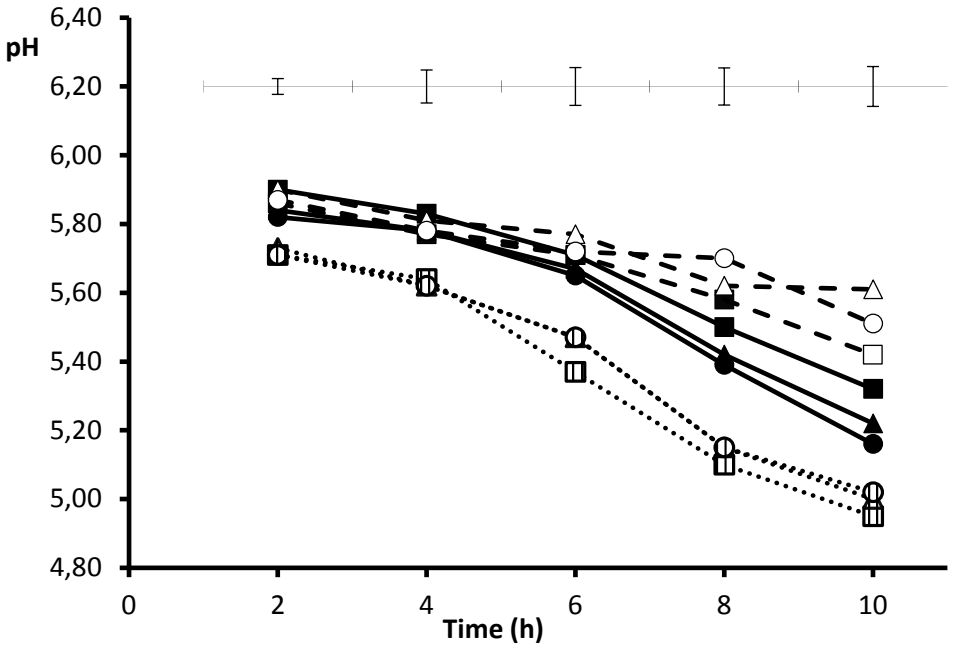
465 [1]: M1, M2 and M3 are varieties of maize; S1, S2 and S3 are varieties of sorghum; B1, B2 and B3 are
466 varieties of barley

467 [2]: BCFA: branched-chain fatty acids (sum of isobutyrate + isovalerate)

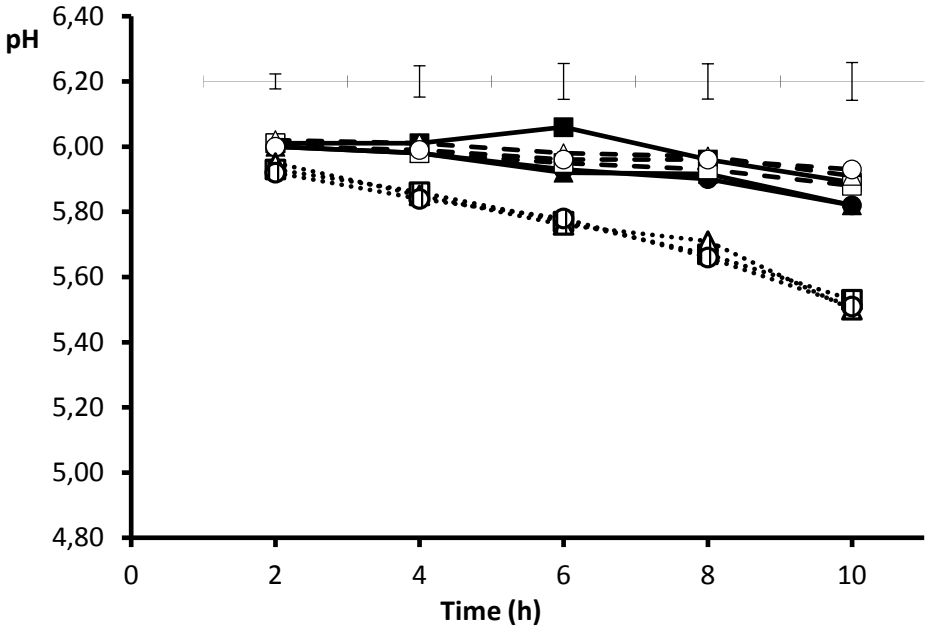
468 [3]: SEM: standard error of the means

Figure 1. Pattern of incubation pH according to the different varieties of maize (M1 ■, M2 ▲, M3 ●, solid line), sorghum (S1 ■, S2 ▲, S3 ●, dashed line) and barley (B1 ▥, B2 ◮, B3 ◍; dotted line) when incubated in a low buffered medium with inoculum from a concentrate diet (CI, Fig. 2a) or from a forage diet (FI, Fig. 2b). Upper bars show standard error of means.

a)

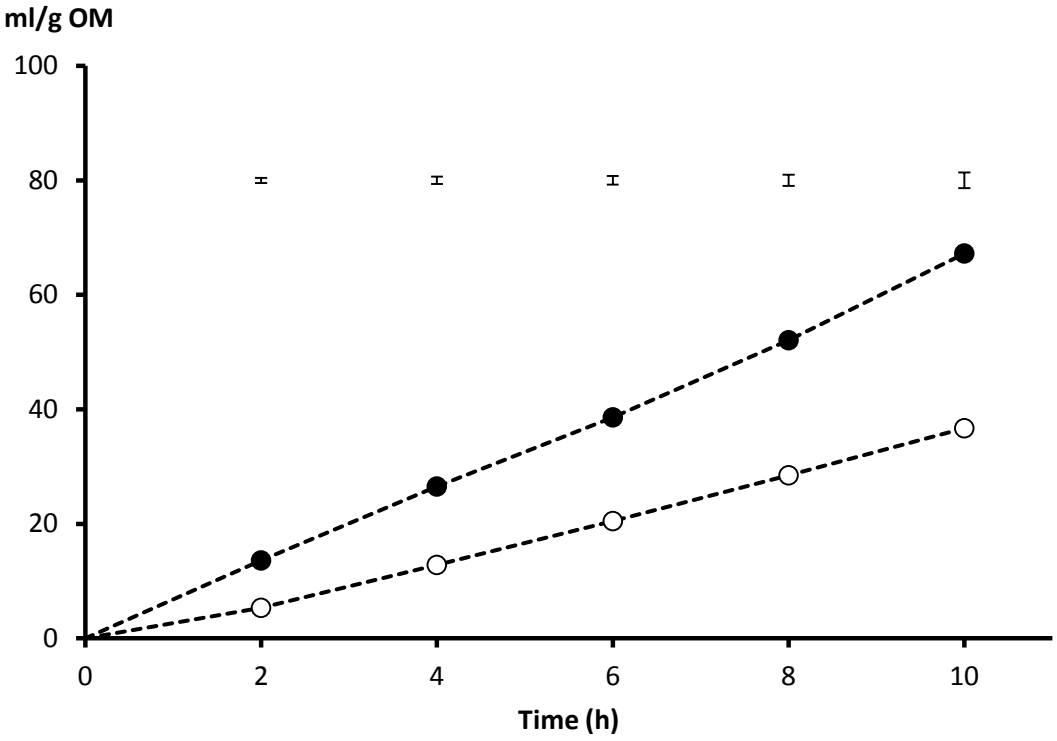


b)



478 **Figure 2.** Average pattern of gas production from the substrates incubated in a low buffered
479 medium with inoculum from a concentrate diet (CI, ●) or a forage diet (FI, ○). Upper bars
480 show standard error of means.

481

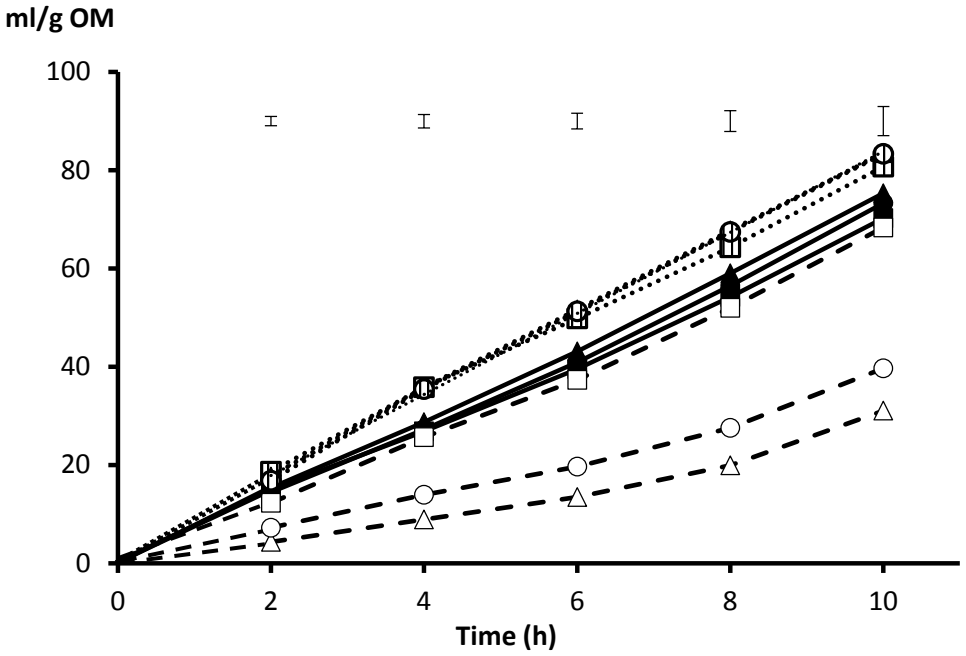


482

483

Figure 3. Pattern of gas production from the different varieties of maize (M1 ■, M2▲, M3●, solid line), sorghum (S1 □, S2 △, S3 ○, dashed line) and barley (B1 ▥ B2 ◮, B3 ◍; dotted line) when incubated in a low buffered medium with inoculum from a concentrate diet (CI, Fig. 3a) or from a forage diet (FI, Fig. 3b). Upper bars show standard error of means.

a)



b)