**Abstract**

Soluble sugars are thought to play an important role in the fermentation processes of the rumen but their actual fermentation rate has not been fully assessed. Some sugars are also used as markers to assess gut permeability in monogastrics but their use in ruminants can be compromised by the hydrolytic activity of rumen microflora. This study aimed to evaluate the fermentability of some naturally occurring and synthetic soluble sugars. The synthetic soluble sugars were included to verify their possible use as markers for studies of gut permeability in ruminants. *In vitro* gas and volatile fatty acid (VFA) production from glucose, fructose, xylose, galactose, sucrose, lactose, arabinose mannitol, lactulose and sucralose were measured in a 24 h-incubation trial using ruminal fluid from heifers adapted or not-adapted to additional sugars in the diet, and with caecal content as inocula. Gas production from the same sugars was further evaluated in a 72 h-incubation trial with not-adapted rumen fluid only. Gas and VFA production were not affected by feeding additional sugars, but significant effects of inocula (ruminal vs caecal), sugars and their interaction were observed. Caecal inoculum produced less gas but higher VFA than ruminal inocula. Fructose and glucose had the highest rates of gas production (10.57% h⁻¹ and 10.42% h⁻¹, respectively), and lactulose and mannitol the lowest (3.47% h⁻¹ and 4.63% h⁻¹, respectively) when fermented with ruminal fluid. Sucralose seemed to have a negative effect on microbial fermentations. Our results indicate that lactulose and mannitol might largely escape rumen fermentation, suggesting their possible use as markers to test gut permeability also in ruminants. This needs to be verified *in vivo*.

**Introduction**

In most common components of dairy cow diet, sugar content ranges from less than 1% to over 10% of dry matter (DM) (Van Soest, 1994; Hoover and Miller-Webster, 1998). The most abundant sugars in plants are: i) glucose and fructose, that can also be found in their free form (Bailey, 1962; Hall, 2002; Buxton and O’Kiely, 2003); and ii) xylose, galactose and arabinose, that are components of hemicelluloses and gums (Fahey and Berger, 1988). Free sugars, such as mono-, di- and oligosaccharides, often account for 5-3% DM or less in dairy cow diets; a range that is also considered the optimum feeding rate (Firkins, 2010). Over recent years, there has been an increasing interest in the possible use of some agro-industrial by-products, such as molasses and milk whey, for ruminant feeding. They are particularly rich in sucrose and lactose, respectively (Hall, 2002; Succi et al., 2002), and can significantly increase sugar content in the diet. To enhance the feed efficiency in ruminant livestock, carbohydrate and protein availability in the rumen should be accurately balanced (Hoover and Stokes, 1991). High-producing dairy cows and fast finishing beef cattle require highly digestible diets, but excessive or too rapid VFA production can impair rumen and gut function (Morgante et al., 2007; Emenark, 2008; O’Grady et al., 2008). Sugars are considered the fastest fermented carbohydrate fraction (Hall, 2002) and are consequently extensively utilized within the rumen, but their actual rate of fermentation has not been fully assessed. Reported values for their fermentation rate in the literature range from approximately 10% h⁻¹ (Doane et al., 1998) up to 700% h⁻¹ (Weisbjerg et al., 1998). According to the results of Molina (2002), in The Cornell Net Carbohydrate and Protein System (CNCP56.0), sugar fermentation rate was recently drastically reduced from 300-500% h⁻¹ to 40-60% h⁻¹ (Van Amburgh et al., 2010). Both rate and molar proportion of VFA produced in the rumen differ significantly depending on the specific sugar fermented and on diet composition (Sutton, 1969). VFA profile affects the efficiency of energy utilization (Orskov and MacLeod, 1993), animal health and performance (Miettinen and Huhtanen, 1996). In particular, butyrate is known to affect reticulo-ruminal epithelial blood flow (Storm et al., 2011), rumen papillae development (Sakata and Tamate, 1979), but also to regulate mammalian transcriptome (Davie, 2005). Feeding sugar-rich diets tends to modify the rumen VFA proportion, with an increase in butyrate concentration (Strobel and Russel, 1986; Oba, 2011). Substituting sugars for starch at low levels in rations for lactating cows has been shown to have a positive effect on dry matter intake (DMI) and milk yield (Broderick et al., 2000), whereas high levels of dietary sugars can lead to rumen pH depression and increased lactate yield (Khalili and Huhtanen, 1991). Besides their role as nutrients, combinations of monosaccharides (mannitol or D-xylose) and disaccharides (lactulose or cellobiose) resistant to mammalian digestive enzymes have been used in humans (Cox et al., 1999; Dastych et al., 2008) and laboratory animals (Hall, 1999) as markers to assess intestinal permeability. Sucralose has been recently proposed as an alternative to lactulose and cellobiose (Melichar et al., 2008) for the same purpose. In adult ruminants, their use may be compromised by the hydrolytic activity of the rumen microflora, but in the literature no data are available on the rumen fermentability of lactulose, mannitol and sucralose. This makes it difficult to evaluate their suitability as markers to assess gut permeability in animals with a fully developed rumen.

The aim of the present work was to improve knowledge of the rate of rumen fermentability of some of the main sugars naturally occurring in feedstuffs and of some synthetic sugars that could be used as markers of gut permeability in ruminants.

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Materials and methods

All of the procedures involving animals were conducted in accordance with the Italian laws on animal experimentation and ethics (Italian Regulation, 1992).

In vitro fermentations

Substrates and inocula

Ten sugars were evaluated for their microbial fermentability. Analytical grade D(+)-glucose, D(+)-fructose, D(+)-xylose, L(+)-arabinose and D-mannitol from Fluka (Buchs SG, Switzerland), and pharmaceutical grade lactulose and sucralose from Acef (Florenzuola d’Arda, Italy).

The ruminal inoculum was obtained 6 h after the morning meal from 4 rumen-cannulated dairy heifers fed a ration based on grass hay and concentrate (85:15, DM basis) and suplemented with rumen fluid from the same heifers not adapted (RuNoAd) to eating additional sugars, or with caecal content (Caec). Adaptation involved supplying 2 heifers daily with hand shaking after each pressure reading. Fermentation bottles without substrate and with hand shaking after each pressure reading. Fermentation bottles without substrate were also included. Sugars and blanks were included.

Fermentation technique

In vitro fermentations were carried out in two trials using the reading pressure technique according to Mauricio et al. (1999) with some modifications. Goering and Van Soest (1970) medium (20 mL) was added by an automatic dispenser (Optifix, Fortuna) to each serum bottle that was purged with oxygen free nitrogen with a butyl rubber stopper and transferred into the vial and injected in the chromatograph.

Trial 1: short-lasting in vitro fermentation with different inocula

In Trial 1, substrates (0.3 g/125 mL glass bottle) were inoculated with ruminal fluid obtained from 4 dairy heifers adapted (RuAd) or not-adapted (RuNoAd) to eating additional sugars, or with caecal content (Caec). Adaptation involved supplying 2 heifers daily for a week before sampling with 0.15 kg of an equal mixture of the ten sugars used as substrate, equal to approximately 2% of the basal (RuNoAd) diet DM.

Incubation lasted 24 h and gas pressure was measured at 1, 2, 3, 4, 5, 7, 9, 12, 20 and 24 h post-inoculation. At the end of this period, the serum bottles were cooled in chilled water to stop fermentation and opened. A portion of the liquid was centrifuged 3000 g for 10 min at 10°C and the supernatant was stored at -20°C for VFA analysis.

Trial 2: long-lasting in vitro fermentation with rumen inoculum

In Trial 2, the same substrates were incubated with rumen fluid from the same heifers not supplemented with sugars (RuNoAd diet). The fermentation was carried out as in Trial 1 but was extended to 72 h with additional readings at 36, 48 and 72 h post-inoculation.

Volatile fatty acid analysis

Volatile fatty acid was quantified using a gas chromatograph (model 7820A GC, Agilent Technologies, Santa Clara, CA, USA) equipped with a DB-FAP capillary column (30 m x 250 m x 0.25 m; Agilent J&W GC column) and a flame-ionisation detector. The oven temperature was held for 5 min at 10°C and then increased by 5°C/min to 140°C. Injector temperature was 250°C, while detector temperature was 300°C. Analysis time was approximated 21 min. The samples were dosed by an auto-sampler at an injection size of 1 µL using the split method with 25:1 splitting ratio. Hydrogen and air were used for flame ionisation detection. Carrier gas was nitrogen with a constant flow of 1.78 mL/min. Pivalic acid was used as internal standard. For the gas chromatographic (GC) analysis, the samples were prepared as follows: 1 mL of oxalic acid (0.12 M) and 1 mL of pivalic acid solution (1 g pivalic acid + 50 mL formic acid, made up to 1 L with distilled water) were added to 2 mL of sample. After centrifugation (3000 g for 10 min at 10°C) the clear supernatant was transferred into the vial and injected in the chromatograph.

Calculations and statistical analyses

The raw gas production values from each run were corrected for gas released from blanks and reported gas values were expressed as mL/g monosaccharide. To do this, we divided the initial weight of the disaccharides by 1.05. Corrected values were fitted with time to the exponential model (Orskov and McDonald, 1979) Y = b (1-e (-c t)) where Y is the volume of gas production (mL/g monosaccharide) at time t, b is the potential gas production, c is the fermentation rate (h⁻¹), and t is the incubation time (h). The parameters b and c were estimated by an iterative least-squares procedure using the PROC NLIN of the SAS software (release 8.02, SAS Inst. Inc., Cary, NC, USA). VFA concentrations measured in the theses with substrate were corrected for the corresponding average values measured in the blanks. Data relative to volumes and kinetic parameter of gas production and VFA were statistically evaluated by ANOVA using the GLM procedure of SAS (release 8.02, SAS Inst. Inc., Cary, NC, USA), considering the factors: type of inoculum (n=3), type of substrate (n=10), and their interaction. P<0.05 was considered significant.

Results

Trial 1: short-lasting in vitro fermentation with different inocula

Gas production

Cumulative gas volumes obtained in Trial 1 were fitted to the exponential model. This showed that the duration of the incubation was not sufficient to obtain a correct estimation of the plateau and some aberrant values were obtained for the kinetic parameters. The amount of gas cumulatively produced was consequently evaluated at three fixed times: 3h, 9h and 24h. Data of volumes of gas produced are shown in Table 1. A significant effect of the inocula was evident at all times, with Caec leading to lower (P<0.01) volumes (21.79, 173.58 and 266.71 mL/g at 3, 9 and 24h, respectively) compared with adapted (52.37, 199.76 and 295.09 mL/g) or not-adapted (52.24, 203.49, 295.80 mL/g) rumen fluid. No significant differences were found between RuAd and RuNoAd at any time point. Gas production from sucralose was very low or even negative, i.e.
lower than the blanks, at all times.

Glucose, fructose and sucrose produced the highest volumes of gas (100-110 mL/g) and lactulose and mannitol the lowest (18.89 and 21.75 mL/g, respectively) with ruminal inocula after 3 h of fermentation. At the same time, with caecal inoculum, the highest gas yield was recorded for glucose (58.29 mL/g) and lactose (56.70 mL/g), whereas lactulose and mannitol produced the lowest volumes (8.48 and 3.02 mL/g, respectively). The same ranking of fermentability for RuAd and RuNoAd was recorded after 9 h of incubation: fructose, glucose, and sucrose yielded the highest gas (270 to 280 mL/g) but lactulose and mannitol produced less than 150 mL/g. With Caec the substrates can be divided into three groups: glucose, lactose and galactose had the highest gas yields (250 to 280 mL/g), mannitol and lactulose the lowest (35 to 80 mL), and the other sugars had intermediate yields. At 24 h the differences among sugars were less pronounced with RuAd and RuNoAd, but with Caec the highest gas yields were recorded for glucose, galactose and arabinose (315 to 325 mL/g), whereas sucralose, mannitol and lactulose produced the lowest amount of gas (230 to 275 mL/g).

Volatile fatty acids

Concentrations of VFA, after 24 h in vitro fermentation of different sugars with different sources of inoculum are shown in Table 2. Inoculum significantly (P<0.01) affected the amount of VFA production after 24 h of fermentation. Higher concentrations were recorded for Caec compared with ruminal inocula. On average, 52.16 mmol/L were recorded in Caec vs 38.23 mmol/L for rumen fluid. Adaptation to sugars did not influence VFA concentrations. VFA concentration was largely (P<0.01) influenced by substrate, and a significant interaction between inoculum and substrate was also found (P<0.01). Sucralose produced very low amounts of VFA when incubated with Caec (7.77 mmol/L) and even lower than in blanks with rumen fluid (-2.72 and -0.40 mmol/L, for RumAd and RumNoAd, respectively). With Caec, the highest VFA concentration was detected for fructose (62.01 mmol/L) and the lowest, besides sucralose, for xylose (48.83 mmol/L). With rumen fluid, the highest VFA levels were recorded for xylose and arabinose (47.20 and 48.84 mmol/L, respectively); mannitol, sucrose and lactulose produced the lowest VFA levels (40.45, 39.83 and 38.04 mmol/L, respectively).

Relative VFA concentrations, expressed as mol/100 mol of VFA, were influenced by inocula and kind of sugars, as well as their interaction (P<0.01). When sugars were incubated with the caecal inoculum, a higher molar percentage of acetic acid (63.58) was measured but lower propionic acid (21.53) and butyric acid (9.76) compared with rumen fluid (an average 52.15, 27.01 and 15.11 for acetate, propionate and butyrate, respectively), and the non-gluco-genic ratio (NGR) (Orskov, 1975) was also higher in Caec than with rumen fluid (4.14 vs 3.41). The adaptation of the donor animals to sugars did not affect the percentage of propionic acid but modified to some extent (P<0.01) the molar proportion of all the others acids. In particular, RumAd led to a lower proportion of acetate than RuNoAd (51.24 vs 53.01%), but to a higher percentage of butyric acid (16.27 vs 13.94%) and a higher NGR (3.44 vs 3.38).

**Trial 2: long-lasting in vitro fermentation with rumen inoculum**

In the Trial 2, a 72 h in vitro fermentation with ruminal inoculum from non-adapted heifers was carried out to achieve a better estimation of the kinetic parameters of the gas production. Results are shown in Table 3. When sucralose was the substrate it was not possible to fit the cumulated gas volumes with the adopted model. For the other sugars, pseud-R² was always above 0.98, with the exception of lactulose (Pseudo-R² = 0.95). The parameters descriptive of the gas production pattern were significantly affected by the kind of sugar (P<0.01). Estimated potential gas yield (h) ranged from 368.43 mL/g for fructose to 436.30 mL g for lactulose, whereas fermentation rate (c) ranged from 3.47%/h for lactulose to 10.57%/h for fructose. Fermentation rate for glucose and fructose was 10%/h; this was very similar to sucrose (9.8%/h) although the difference was significant. Arabinose, xylose and galactose had similar rates (an average 7.0%/h), whereas mannitol also had a fermentation rate as low as 4.63%/h.

Table 1. In vitro gas production (mL/g of dry matter) obtained after 3, 9 and 24 h of fermentation of different sugars incubated with different sources of inoculum.

| Sugar   | 3 h     | 9 h     | 24 h     |
|---------|---------|---------|----------|
|         | Caec    | RuNoAd  | RuAd     | Caec    | RuNoAd  | RuAd     | Caec    | RuNoAd  | RuAd     |
| Glucose | 58.29   | 110.19  | 105.66   | 280.26  | 277.97  | 274.84   | 323.64  | 339.25  | 333.08   |
| Fructose| 13.03   | 104.94  | 98.62    | 211.12  | 280.11  | 276.10   | 302.48  | 340.30  | 342.84   |
| Xylose  | 10.92   | 38.29   | 38.42    | 183.00  | 212.14  | 223.86   | 308.16  | 330.38  | 339.25   |
| Galactose| 35.95  | 43.04   | 42.46    | 249.16  | 250.50  | 256.02   | 314.13  | 333.97  | 345.44   |
| Arabinose| 13.39  | 50.05   | 51.50    | 214.93  | 240.32  | 234.61   | 319.97  | 340.12  | 330.88   |
| Mannitol| 3.02    | 21.11   | 22.38    | 36.06   | 146.71  | 151.00   | 228.02  | 298.45  | 295.43   |
| Sucrose | 17.91   | 105.76  | 116.27   | 228.07  | 273.61  | 263.68   | 297.35  | 336.83  | 324.65   |
| Lactose | 56.70   | 34.40   | 31.68    | 254.67  | 211.22  | 224.77   | 298.53  | 320.05  | 311.92   |
| Lactulose| 8.48   | 19.18   | 18.59    | 78.27   | 109.42  | 134.37   | 276.20  | 315.86  | 340.48   |
| Sucralose| 0.23   | -3.26   | -3.16    | 0.24    | -4.42   | -4.31    | -1.32   | -4.30   | -3.98    |
| SEM     | 16.56   | 90.32   |          |         |         |          | 77.19   |         |          |

Caec, caecal inoculum; RuAd, ruminal inoculum from animals adapted to feed with sugars; RuNoAd, ruminal inoculum from animals not adapted to feed with sugars.
Discussion

Trial 1: short-lasting in vitro fermentation with different inocula

The results obtained clearly show differences in the fermentation patterns among the different sugars and the sources of inoculum. Differences attributable to inoculum were more evident during the first part of fermentation, at 3 and 9 h, but tended to decrease at 24 h. This is consistent with the observations of Mauricio et al. (1998) and Bani et al. (1999) who reported a slower initial activity of faecal inoculum compared with rumen fluid. This could reflect the need for an adaptation period for caecal and faecal microbes to allow the selection of a microbial population better adapted to the utilization of the specific substrates and not only a general increase in the number of bacteria. In fact, in preliminary trials, the simple increase of the amount of faecal inoculum did not reduce the differences compared with rumen fluid. With sucralose, gas production and VFA concentration were lower than in the blank, revealing that this substrate was not available to caecal or ruminal microbes but it could also have a slight antimicrobial effect, as already demonstrated in vitro against periodontal pathogens (Prashant et al., 2012). Among the other substrates, the monosaccharides glucose and fructose and the corresponding disaccharide sucrose had earlier (3 h) availability to microbial fermentation than all the other sugars used in the experiment, whereas mannitol and lactulose appeared to be the least susceptible sugars to microbial digestion. Czerkawski and Brekenridge (1969) also found a higher fermentability of glucose compared with galactose, xylose and ribose. In a short fermentation (2 h), Sutton (1968) observed a higher VFA production from glucose, fructose and sucrose compared to galactose, xylose and arabinose when rumen inoculum was obtained from cows fed a high-forage diet, similar to that used in the present experiment, but not when a high-concentrate diet was fed to donor animals (Sutton, 1969). At an intermediate time (9 h), a similar situation was recorded, but galactose recovered the low initial fermentability reaching values of gas production similar to glucose, fructose and sucrose. On the other hand, lactose appeared fermented to a lower extent than its monosaccharide components, galactose and glucose. Although disaccharide hydrolysis did not appear to be a limiting step for further rumen fermentation (Weisbjerg et al., 1998), Bond et al. (1998) reported a lower growth rate of S. bovis on lactose versus glucose. The gas yield at this time can be assumed to be similar to liquid mean retention time in the rumen of dairy cattle (Hristov et al., 2003). Based on this, it was evident that both lactulose and mannitol were fermented to a much lower extent compared with all the other substrates and, consequently, a considerable amount can escape rumen fermentation when orally dosed. Sucralose

Table 2. Production of volatile fatty acids (VFA, mmol/L) and their molar percentages (%:mmol/100 mmol of total VFA) after 24 h in vitro fermentation of different sugars with different sources of inoculum.

| Inoculum | Sugars | SEM | Significance |
|----------|--------|-----|--------------|
|          | Glucose Fructose Xylose Galactose Arabinose Mannitol Sucrose Lactose Lactulose Sucralose |       |              |
| VFA, mmol/L |        |     |              |
| Caec      | 57.82  62.01 48.83 58.43 54.41 54.98 59.87 57.78 59.67 7.77 |       |              |
| RuAd      | 40.42  41.57 47.24 44.61 47.61 39.88 37.98 42.56 37.84 2.72 | 10.18 | 0.01 0.01 0.01 |
| RuNoAd    | 42.94  43.77 47.16 43.70 50.07 41.02 39.88 39.54 38.23 2.72 |       |              |
| Acetate, %|        |     |              |
| Caec      | 64.01  61.87 64.52 65.62 60.22 61.35 64.01 71.28 69.11 53.79 |       |              |
| RuAd      | 47.44  47.29 52.92 50.14 53.09 44.28 47.26 53.84 54.99 61.23 | 0.13  | 0.01 0.01 0.01 |
| RuNoAd    | 49.38  49.09 55.68 52.25 54.66 43.77 48.48 55.04 57.49 64.59 |       |              |
| Propionate, %|       |     |              |
| Caec      | 19.16  23.77 21.16 18.95 23.46 26.58 20.63 15.87 19.98 24.16 |       |              |
| RuAd      | 27.02  27.43 29.64 27.44 28.87 33.00 27.55 28.95 23.17 17.57 | 0.16  | 0.01 0.01 0.01 |
| RuNoAd    | 28.48  29.55 28.35 27.99 28.37 33.48 28.58 26.96 22.99 14.76 |       |              |
| Butyrate, %|       |     |              |
| Caec      | 12.24  8.28 10.00 11.01 11.41 7.02 10.92 8.72 6.82 11.22 |       |              |
| RuAd      | 20.54  20.26 12.73 17.07 12.77 16.98 20.22 12.25 17.16 12.73 | 0.20  | 0.01 0.01 0.01 |
| RuNoAd    | 16.35  15.55 10.45 14.02 11.00 16.25 17.32 12.56 14.60 11.31 |       |              |
| Valerate, %|       |     |              |
| Caec      | 1.96   2.04 1.90 1.93 2.09 2.06 1.95 1.88 1.83 4.20 | 3.43  | 0.03 0.01 0.01 |
| RuAd      | 2.99   3.10 2.54 3.00 2.81 3.76 2.99 2.95 2.75 4.34 | 0.07  | 0.01 0.01 0.01 |
| RuNoAd    | 2.77   2.79 2.58 2.82 2.79 3.57 2.71 2.79 2.54 3.98 |       |              |
| Isobutyrate, %|       |     |              |
| Caec      | 1.12   0.96 1.05 1.15 1.19 1.15 1.02 1.07 0.91 2.41 | 0.19  | 0.06 0.01 0.01 |
| RuAd      | 0.59   0.55 0.74 0.78 0.87 0.55 0.58 0.60 0.60 1.19 | 0.19  | 0.06 0.01 0.01 |
| RuNoAd    | 1.04   1.01 1.06 1.03 1.13 0.94 0.99 0.98 0.91 1.59 |       |              |
| IsoValerate, %|       |     |              |
| Caec      | 1.50   1.48 1.37 1.45 1.61 1.85 1.47 1.38 1.36 4.21 | 0.07  | 0.01 0.01 0.01 |
| RuAd      | 1.42   1.37 1.41 1.57 1.59 1.42 1.40 1.41 1.33 2.94 |       |              |
| RuNoAd    | 1.99   2.00 1.88 1.98 2.05 1.99 1.92 1.76 1.57 3.77 |       |              |
| NGR       | 4.62   3.09 3.99 4.62 3.54 2.84 4.16 5.59 4.14 3.16 | 0.24  | 0.01 0.01 0.01 |
| RuAd      | 3.28   3.20 2.64 3.07 2.32 2.37 3.18 2.71 3.85 4.93 | 0.09  | 0.01 0.01 0.01 |
| RuNoAd    | 2.88   2.71 2.70 2.57 2.70 2.26 2.91 2.97 3.77 5.91 |       |              |

Caec, caecal inoculums; RuAd, ruminal inoculum from animals adapted to feed with sugars; RuNoAd, ruminal inoculum from animals not adapted to feed with sugars; VFA, volatile fatty acids produced after 24h fermentation (mmol/L); NGR, non glucogenic ratio- (acetate+2 butyrate)/propionate.
remained almost unfermented and it can be considered that an oral dose can totally escape from the rumen and reach the abomasum. The lower gas yield and VFA concentration compared with the blanks containing only the buffered rumen fluid agree with the above-mentioned negative gas yield and a possible negative interference of this sugar on microbial fermentation. This agrees with the antimicrobial effect of sucralose against periodontal pathogens reported by Prashant et al. (2012).

There was a clear effect of the source of inoculum on VFA molar proportions measured at the end of fermentation. With Caec, a higher proportion of acetate and lower propionate and butyrate were measured than with rumen fluid. Váradyová et al. (2005) measured small differences between sheep ruminal and faecal inocula with fibrous substrates but higher acetate and lower propionate when the highly fermentable barley grain was the substrate. Cutrignelli et al. (2005) did not find any significant differences when incubating different feedstuffs with faecal and ruminal inoculum from buffalo. It seems evident that there is an interaction of inoculum x substrate, a likely consequence of the different composition of the microbial population present in rumin liquor and in the caecal digesta (Omed et al., 2000). Adaptation to sugars did not influence the amount of VFA produced but had a moderate though significant effect on VFA molar proportions, decreasing the proportions of acetate and butyrate and increasing the proportions of propionate. The small amount of additional sugars fed to the heifers (0.15 kg head⁻¹ d⁻¹), chosen to keep the total dietary sugar content within normal ranges, has likely contributed to the lack of major effects on fermentations. Comparing mono- and disaccharides it was not possible to identify any clear difference in the molar percentages of VFA measured at the end of the fermentation, in agreement with Czerkawski and Breckenridge (1969) who obtained similar proportions of VFA incubating glucose, fructose or sucrose. In addition, Heldt et al. (1999) found different proportions of VFA between monosaccharides and sucrose when incubated in vitro with low but not with high levels of degradable protein, suggesting an interaction between sugars and availability of a source of amino acids. The medium used in the present experiment contained Trypticase™ Peptone as source of soluble peptides. Sutton (1968, 1969) reported a marked effect of substrate on VFA molar proportion when different sugars were individually fermented in vitro for a short time (2 h). However, the ranking of the different sugars for acetate, propionate and butyrate molar percentage changed according to the fermentability of the diet fed to the donors’ animals and the amount of substrate fermented. It appears evident that the composition of VFA produced by rumen microbial fermentation is affected by the specific sugars fermented, but extrapolating the results of in vitro trials to in vivo conditions can be misleading, as the experimental conditions have a major effect on the products of in vitro fermentation.

**Trial 2: Long-lasting fermentation**

Based on the non-significant effect of animal adaptation to eating sugars on the gas production, and the incomplete achievement of a defined plateau observed in Trial 1, a longer (72 h) incubation was carried out with the same substrates using a ruminal inoculum from non-adapted animals.

The calculated fermentation rates (k₀), ranging from 3.47% h⁻¹ for lactulose to 10.57% h⁻¹ for fructose are clearly lower than the values generally assumed for sugar digestion in the fore stomach. In rumen and duodenal fistulated dairy cows, Weisbjerg et al. (1998) estimated a rate of fermentation for glucose, fructose and galactose ranging approximately 300-700% h⁻¹. Similarly, in the CNCPs model, fermentation rate of simple sugars (A2 fraction) was initially set at 300-500% h⁻¹, but recently (Lanzas et al., 2007) it was dramatically lowered to 40-60% h⁻¹, based on in vitro results obtained by Molina (2002) by incubating sugars in combination with a source of fibre. In spite of this, many other papers have reported values more similar to those obtained in the present experiment. Doane et al. (1998) reported a fermentation rate as high as 11.8% h⁻¹ for the rapid fraction of freeze-dried, unfermented forages. Calabrò et al. (2005) measured k₀ values of 16.3 and 16.5 % h⁻¹ for the CNCPs A fraction of fresh oats and oat hay, respectively. Azarfar et al. (2007) reported a maximum fractional rate of degradation of 20% h⁻¹ for the soluble washable part of different grains. From the results of Mould et al. (1999), when glucose is incubated alone, a k₀ of approximately 5% h⁻¹ can be estimated.

It appears evident that the experimental procedures can greatly affect the estimation of sugar fermentation rate and use of a standard methodology is recommended. Besides the absolute values of fermentability, our data confirm that there are relevant differences among sugars with regard to their fermentation rate, as already outlined by Sutton (1968, 1969). Glucose, fructose and the correlated disaccharide sucrose showed the highest fermentation rate, as often reported. On the contrary, mannitol and lactulose were fermented at a low rate, and sucralose was found to be almost unfermentable. The low rumen fermentability of the naturally occurring mannitol and of the two tested synthetic disaccharides, lactulose and sucralose, might suggest that, when orally administered, a significant amount can escape rumen digestion and reach the duodenum. This highlights the possibility of using these sugars as markers to assess gut permeability also in animals with a fully functional rumen, but this needs to be confirmed in vivo. Sucralose appeared almost unfermentable by rumen microbes and in this regard it would be even superior to lactulose and mannitol as a sugar probe. Nevertheless, it induced a lower gas production than in the control fermenters where no substrate was added. This could indicate a negative effect on rumen microbial metabolism, a hypothesis that requires further investigation.

| Sugar      | Potential gas production, mL/g DM | Fermentation rate, %/h |
|------------|----------------------------------|------------------------|
| Glucose    | 372.03                           | 10.42                  |
| Fructose   | 368.83                           | 10.57                  |
| Xylose     | 378.07                           | 6.79                   |
| Galactose  | 374.73                           | 7.22                   |
| Arabinose  | 382.20                           | 7.18                   |
| Mannitol   | 385.40                           | 4.63                   |
| Sucrose    | 368.43                           | 9.83                   |
| Lactose    | 379.03                           | 6.08                   |
| Lactulose  | 436.30                           | 3.47                   |
| SEM        | 76.11                            | 0.000000804            |
| Significance | Sugars 0.01                      | 0.01                   |

DM, dry matter.
Conclusions

Mono- and disaccharides differ in their rumen fermentation rates and they also have a different availability for microbial inocula obtained from caecum or rumen. In addition to fermentation rates, inocula and the kind of sugars can affect also the proportions of VFA originating from their microbial utilization with possible consequences not only on animal metabolism but also on ruminal or large bowel mucosa. Absolute $k_d$ values can be largely influenced by experimental techniques and there is a need to achieve a standard methodology validated against in vivo trials.

Rumen microbes do not ferment sucralose. However, further research is needed to evaluate whether or not sucralose adversely affects rumen microflora. Lactulose and mannitol are fermented to a significantly lower extent in the rumen compared to the most common sugars found in feedstuffs. Both could be valuable as markers to test the integrity of the digestive mucosa in full ruminants, as has already been successfully performed in monogastric or pre-ruminant veal calves. However, further in vivo evaluation studies are required.

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