In vitro fermentation and acidification potential of several carbohydrates sources used in concentrate-based diets for growing ruminants

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In vitro fermentation and acidification potential of several carbohydrates sources used in concentrate-based diets for growing ruminants

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Abstract. This *in vitro* work aims to study how the different carbohydrate sources fed during the fattening of young ruminants can modulate the characteristics of ruminal fermentation. Six carbohydrate feeds (barley, B; maize, M; sorghum, S; sugarbeet pulp, BP; citrus pulp, CP; and wheat bran, WB) were tested in an *in vitro* semi-continuous culture system under a poorly buffered medium from 0 to 6h, and allowed pH to rise to around 6.5 from 8 to 24h. Rumen fluid was obtained from three lambs fed *ad libitum* on a diet composed of concentrate and barley straw. The pH from 2 to 12h was reduced at a higher extent with CP, which recorded its minimum value at 6h (5.60; P<0.05), but recovered thereafter to 6.63 at 24h. During the whole incubation period, the volume of gas recorded with CP was the highest (P<0.05), followed by B and WB, whereas the lowest volume was recorded by S. Gas production results were supported by dry matter disappearance (DMd) at 24h. Concentration of volatile fatty acids (VFA) and lactic acid was the highest with CP (P<0.05), followed by WB and B. A higher acetate proportion was observed with BP and CP (P<0.05) that recorded the lowest butyrate proportion. Regarding microbial diversity, after 8h, within each incubation series substrates clustered together, except for CP and WB. CP acidified the incubation medium in a higher extent, followed by WB and B. The capacity of substrate acidification plays an important role on dynamics of *in vitro* microbial fermentation.

Keywords. Carbohydrates – Ruminal fermentation – Gas production – pH – *In vitro* semi-continuous culture system.

Fermentation* in vitro *et potentiel d'acidification de quelques sources de glucides utilisées dans les régimes à base de concentrés pour les ruminants en croissance

Résumé.*Ce travail* in vitro *avait pour objectif d'étudier comment les différentes sources de glucides utilisées lors de l'engraissement des jeunes ruminants peuvent moduler les caractéristiques de la fermentation ruminale. Six aliments glucidiques (orge, B; maïs, M; sorgho, S; pulpe de betterave, BP; pulpe d'agrumes, CP; et son de blé, WB) ont été testés dans un système in vitro de culture semi-continue sous un milieu faiblement tamponné de 0 à 6h, et ajusté à un pH d'environ 6,5 á partir de 8h. Le liquide ruminal provenant de trois agneaux recevant ad libitum une ration composée de concentré et de paille d'orge . De 2 á 12h, le pH a fortement chutéavec CP qui a enregistré la plus faible valeur á 6h (5,60; P<0,05), mais ensuite le pH a augmenté pour atteindre 6.63 á 24h. Durant toute la periode d'incubation, le volume de gas enregistré avec CP a été le plus élevé (P<0,05), suivi par B et WB, alors que le volume le plus bas a été enregistré avec S. Les résultats de la production de gaz ont été soutenus par la disparition de la matière sèche (DMd) á 24h. Les concentration des acides gras volatiles (VFA) et d'acide lactique ontété supérieures avec CP (P<0,05), suivi par WB et B. Une proportion élevée d'acétate a été observée avec BP et CP (P<0,05) qui ont enregistré la proportion la plus basse de butyrate. En ce qui concerne la diversité microbienne, après 8h, au sein de chaque série d'incubation les substrats ont été regroupés, à l'exception du CP et WB. La CP a acidifié davantage le milieu d'incubation, suivi par WB and B. La capacité d'acidification du substrat joue un rôle important dans la dynamique de la fermentation microbienne* in vitro.

Mots-clés. *Glucides – Fermentation ruminale – Production de gaz – pH – Système* in vitro *de culture semi-continue.*

I – Introduction

A wide array of carbohydrate sources, varying in composition, is currently used as energy sources in ruminant diets. Cereal grains are high starch sources, which availability differs according to its chemical structure, protein matrix or presence of phenolics (O´Brien, 1999; Offner *et al*., 2003), whereas other substrates are fermentable fibre sources, with either insoluble (cellulose, hemicelluloses) or soluble (mostly pectin) polysaccharides, and also containing variable proportions of either starch or sugars. Fitting substrate characteristics to the fermentative ability of rumen microbiota, when the environmental conditions are maintained under an optimal range is a key factor for maximising energy utilisation in ruminants, thus the risk of physiological impairment is reduced. Whereas their study *in vivo* is often biased by hardly controlled fermentation conditions such as passage rate, *in vitro* techniques are widely used for obtaining a good insight into rumen fermentation processes. However, most of these *in vitro* methods are designed for mimicking high forage diets, and it is not easy to adapt some main physiological conditions such as pH and rate of passage to the environment promoted by high concentrate diets. Using a simple semi-continuous incubation system (Fondevila and Pérez-Espés, 2008), and applying the procedure proposed by Amanzougarene and Fondevila (2018) for controlling incubation pH, in this work several carbohydrate sources with varying composition were compared in terms of ruminal fermentation pattern, simulating the rumen pH and the liquid outflow rate to conditions of intensive feeding systems.

II – Materials and methods

Six carbohydrate sources, namely three cereal grains, barley (var. Gustav, B); maize (var. Dekalb 6667Y, M); and a brown sorghum (S) and three fibrous feeds, sugarbeet pulp (BP); citrus pulp (CP); and wheat bran (WB) were tested in three incubation series of 24h. Substrate samples (800 mg) were dispensed into nylon bags (45 µm pore size) that were sealed and introduced in duplicated bottles filled under CO_2 flux with 80 mL of incubation solution including 16 mL thawed inoculum (0.20 of total volume). As donor of rumen inoculum, three growing lambs weaned at 7 weeks ± 8 days were fed *ad libitum* for 35 days to obtain the adequate inoculum characteristics, and then were slaughtered. A ration was composed by a standard concentrate (barley, maize, wheat, and soybean meal) plus barley straw. Concentrate and straw were fed *ad libitum* allowing for 0.10 daily refusals. The rumen contents of each animal were filtered through a cheesecloth, immediately frozen in liquid nitrogen and preserved at –80 ºC until using. Immediately before incubation, rumen inoculum was thawed in a water bath at 39ºC. Two buffer solutions were made up, one with 0.006 M bicarbonate in order to get a poorly buffered medium pH (≈5.5) for being used from 0 to 6h incubation, and another with 0.058 M bicarbonate to fitting medium pH around 6.5 for being used from 8 to 24h incubation (Amanzougarene and Fondevila, 2018). Pressure produced on each bottle was measured every 2h (from 0 to 12h) or every 4h (from 12 to 24h), and gas volume was expressed per unit of incubated organic matter (OM). Along the incubation, a volume of liquid medium was extracted immediately after gas measurement, and replaced anaerobically by the same volume of incubation solution (without microbial inoculum) to simulate an approximate liquid turnover rate of 0.08/h. Incubation pH was recorded on every extraction, and medium was sampled at 6, 10 and 24h and immediately frozen until determination of volatile fatty acids and lactic acid concentrations, or sampled at 8h and frozen in liquid nitrogen for microbial biodiversity by terminal restriction fragment polymorphism (tRFLP). At the end of incubation, bags with the substrate residue were removed, rinsed and dried at 60ºC for 48h for determination of dry matter disappearance (DMd).

The microbial diversity results were analysed with R statistical software in the form of relative abundance. Results of the different substrates were analysed by ANOVA using the Statistix 10 software package, considering the incubation series as a block (n=3), and the incubated bottles as the experimental unit. The differences were considered significant when $P<0.05$. The Tukey *t* test ($P<0.05$) was used for the multiple comparison between means.

III – Results and discussion

The mean inoculum pH at the start of the incubation series was 6.45 ± 0.15. The lowest pH was recorded at 6h of incubation (5.96 ± 0.19), and afterwards it increased to reach an average of 6.64 ± 0.02 at 24h, showing that rumen pattern can be simulated *in vitro* by changing buffer concentration, as planned (Fig. 1a). Among substrates, from 2 to 12h CP recorded the lowest incubation pH ($P<0.05$), reaching its minimum at 6h (5.60; $P<0.05$), but it recovered thereafter to 6.63 at 24h. S, BP, and M maintained the highest medium pH ($P<0.05$) from 4 to 8h. Regarding the gas production, CP recorded the highest volume throughout all the incubation period (Fig. 2a, $P<0.05$), and the lowest volume was recorded by S. Similar to the gas production results, DMd was highest for CP (0.513), and the lowest DMd was shown with S (0.251; $P<0.05$, SEM=0.0178).

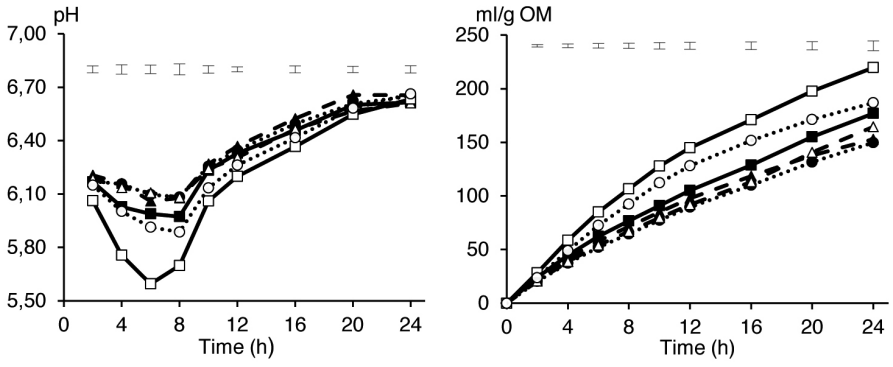

Fig. 1a **Fig. 1b**

Fig. 1. Pattern of incubation pH (Fig. 1a) and gas production (Fig. 1b) (B ■, CP □; solid line, M ▲, BP △; dashed line , S ●, WB ○; dotted line). Upper bars show standard error of means (n=3).

Similarly, these results were supported by those observed in concentration of VFA and lactic acid. Thus, at 6h incubation, the highest concentration of lactic acid was recorded with CP (8.70 mmol/L; $P<0.05$) compared to the other substrates (2.40 mmol/L, on average). A similar trend was observed on the VFA concentration. As it is shown in Table 1 for results observed at 24h,the highest total VFA concentration was recorded by CP, and the lowest was showed by M and S ($P<0.05$). The heterogeneous chemical nature of substrates was reflected through a higher proportion of acetate ($P<0.05$) with BP and CP at the expense of butyrate, probably because of their higher content of rapidly fermentable fibre and pectin. The highest butyrate proportion was recorded with M and the lowest was observed with BP ($P<0.05$) whereas no differences were recorded on propionate proportion. Results from bacterial biodiversity after 8h of incubation (Fig. 2) were markedly affected by the incubation series (that is, the donor animal). Apart of this, within each incubation series substrates clustered together except for CP (series 2 and 3) and WB (series 1), which means that the characteristics of these byproducts might lead to a shift in bacteria diversity.

These results showed that citrus pulp has an acidic capacity of even higher magnitude than the other substrates because of its richness in both soluble fibre and soluble sugars (Barrios-Urdaneta *et al*., 2003). Despite the important proportion of pectin in sugarbeet pulp, a larger drop in medium pH was observed with citrus pulp or even wheat bran. Shahmoradi *et al*. (2015) reported that the sugarbeet pulp could affect positively ruminal pH. Among cereal species, despite the high proportion of starch in maize and sorghum (Amanzougarene *et al*., 2018), the structure of the starch endosperm of these species and the proportion of amylose (Offner *et al*., 2003) makes that, as expected, the starch of barley was degraded faster.

Table 2. Total volatile fatty acids concentration (VFA, mM) and molar proportions (mmol/mmol) of acetate (C2) propionate (C3) and butyrate (C4) at 24h for the different carbohydrates

Sub.	VFA	C2	C3	C4
B	21.4^{ab}	0.512^{b}	0.248	0.161^{ab}
M	14.9^{c}	0.509^{b}	0.239	0.164^{a}
S	17.5^{bc}	0.496^{b}	0.28	0.150^{ab}
BP	19.5^{abc}	0.632^{a}	0.234	0.090^{b}
CP	22.2^{a}	0.616^{a}	0.123	0.205^{ab}
WB	18.4^{abc}	0.517^{b}	0.134	0.266^{ab}
SEM	0.939	0.014	0.0236	0.0148

Means within a column with different superscripts differ (P<0.05).
SEM: standard error of means.

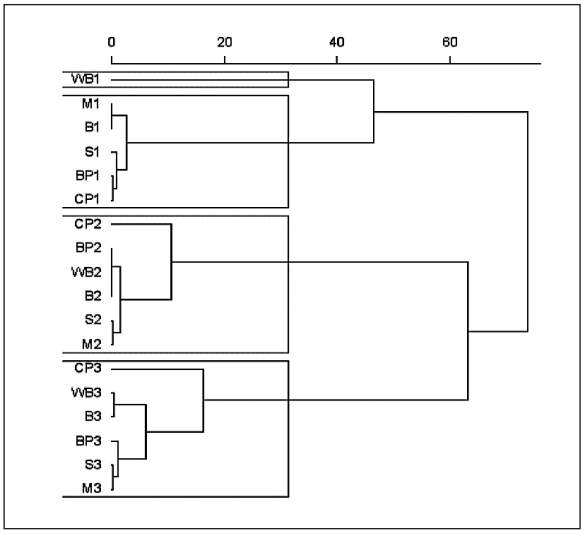

Fig. 2. Dendrogram of bacteria diversity at 8h. Scale bar shows Euclidean distances, "ward method".

IV – Conclusions

Under fermentation conditions of high concentrate feeding, some sources of highly fermentable fibre, such as citrus pulp and at a lower extent wheat bran, may create a more acidic environment than cereal grains. The *in vitro* semicontinuous system may be useful for the study of microbial fermentation of intensive feeding conditions.

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References

Amanzougarene Z and Fondevila M, 2018. Fitting of pH conditions for the study of concentrate feeds fermentation by the *in vitro* gas production technique. *Animal Production Science* 58, 1751-1757.

Amanzougarene Z, Yuste S, Castrillo C and Fondevila M, 2018. *In vitro* acidification potential and fermentation pattern of different cereal grains incubated with inoculum from animals given forage or concentrate-based diets. *Animal Production Science* 58, 2300-2307.

Barrios-Urdaneta A, Fondevila M and Castrillo C, 2003. Effect of supplementation with different proportions of barley grain or citrus pulp on the digestive utilization of ammonia-treated straw by sheep. *Animal Science* 76: 309-317.

Fondevila M and Pérez-Espés B, 2008. A new *in vitro* system to study the effect of liquid phase turnover and pH on microbial fermentation of concentrate diets for ruminants. *Animal Feed Science and Technology* 144, 196-211.

O´Brien L, 1999. Genotype and environment effects on feed grain quality. *Australian Journal of Agricultural Research* 50, 703-719.

Offner A, Bach A, Sauvant D, 2003. Quantitative review of in situ starch degradation in the rumen. *Animal Feed Science and Technology* 106, 81-93.

Shahmoradi A, Alikhani M, Riasi A, Ghorbani G and Gaffari M, 2015. Effects of partial replacement of barley grain with beet pulp on performance, ruminal fermentation and plasma concentration of metabolites in transition dairy cows. *Journal of Animal Physiology and Animal Nutrition* 100, 178-188.