In vitro study of the effect of combinations of cereals and sugar beet pulp on pH and gas production pattern in concentrate or forage-based diets for ruminants

Amanzougarene Z., Yuste S., De Vega A., Fondevila M.

in

Ruiz R. (ed.), López-Francos A. (ed.), López Marco L. (ed.).
Innovation for sustainability in sheep and goats

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 123

2019
pages 83-87

In vitro study of the effect of combinations of cereals and sugar beet pulp on pH and gas production pattern in concentrate or forage-based diets for ruminants

Z. Amanzougarene, S. Yuste, A. de Vega and M. Fondevila*

Depto. Producción Animal y Ciencia de los Alimentos, Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza-CITA, Miguel Servet, 177, 50013, Zaragoza, Spain
*e-mail: mfonde@unizar.es

Abstract. The nature of the dietary carbohydrate fraction may affect fermentative conditions and performance, depending on rumen environment. Three mixtures (1:1 maize:barley, MB, and maize:sugarbeet pulp at either 1:1, MP, or 3:1, 3MP) were incubated in an *in vitro* semi-continuous culture system, using inoculum from lambs receiving a concentrate (CI) or a forage (FI) diet (three 24h series for each inoculum). Medium pH was poorly buffered from 0 to 6h, and allowed to rise around 6.5 from 8h onwards. With CI, minimum incubation pH was reached after 6h, being higher (P<0.05) with MP than with MB and 3MP from then (6.06, 5.97 and 5.95 at 6h, respectively) to 20h (6.78, 6.67 and 6.67). Gas production was highest for MB at 2h and from 6 to 16h, and lowest with 3MP from 2 to 8h, and with MP from 20h onwards (P<0.05), whereas no differences (P>0.05) were recorded neither between MB and 3MP at 20 and 24h, nor between MP and 3MP from 10 to 16h. With FI, pH was lower with MB than with 3MP at 6h (6.33 vs. 6.39, P<0.05), and maintained lowest onwards (P<0.05) with MB. The volume of gas from 3MP was lowest (P<0.05) up to 4h, and lower with 3MP than with MB from 6h onwards (P<0.05), whereas differences between MB and MP were only recorded after 24h. In both concentrate and forage environments, MP maintains a more stable pH pattern while fermentation was not noticeably depressed compared to higher starch proportions mixtures (MB and 3MP).

Keywords. Carbohydrate mixtures – Incubation pH – *In vitro* gas production – Semi-continuous culture system.

Étude* in vitro *de l'effet des combinaisons des céréales et de pulpe de betterave sur le pH et la production de gaz avec des rations à base de concentrés ou à base de fourrage pour les ruminants

Résumé. *La nature des glucides alimentaire peut affecter les conditions et les performances de fermentation, selon l'environnement du rumen. Trois mélanges (1:1 maïs:orge, MB, et maïs:pulpe de betterave soit 1:1, MP, ou 3:1, 3MP) ont été incubés dans un system* in vitro *de culture semi-continue, en utilisant un inoculum provenant d'agneaux recevant une ration á base de concentré (CI) ou une ration á base de fourrage (FI) (trois séries de 24h pour chaque inoculum). Le pH de milieu d'incubation a été faiblement tamponné de 0 à 6h, et il a été tamponné autour de 6.5 à partir de 8h. Avec CI, Le pH minimum d'incubation a été atteint après 6h, à partir de là le pH a été supérieur (P<0.05) avec MP qu'avec MB et 3MP (6.06, 5.97 et 5.95 à 6h, respectivement) jusqu'á 20h (6.78, 6.67 et 6.67). La production de gaz enregistrée avec MB a été supérieure á 2h et de 6 á 16h, et inférieure par rapport à 3MP de 2 á 8h, et avec MP á partir de 20h (P<0.05). Tandis que des différences de production de gaz n'ont pas été enregistrées (P>0,05) ni entre MB et 3MP á 20 et 24h, ni entre MP et 3MP du 10 á 16h. Avec FI, le pH a été inférieur avec MB qu'avec 3MP á 6h (6.33 vs. 6.39, P<0.05), et il a été maintenu inférieur (P<0.05) jusqu'à la fin d'incubation avec MB. Le volume de gaz enregistré par 3MP a été inférieur (P<0.05) á partir de 4h, et inférieur avec 3MP qu'avec MB á partir de 6h (P<0.05), tandis que des différences entre MB et MP ont été enregistrées seulement après 24h. Dans les deux cas, avec CI ou avec FI, MP maintient plus stable le pH du milieu, en revanche sa fermentation n'a pas été sensiblement baissée par rapport aux deux autres mélanges avec des proportions plus élevées d'amidon (MB et 3MP).*

Mots-clés. *Mélanges de glucides – pH d'incubation – Production de gaz* in vitro *– Système semi-continu de culture* in vitro.

I – Introduction

The fattening diets fed to lambs in Southern Europe are characterised by a high rate of cereal grains as the main carbohydrates sources. However, using such feeds promotes a high rate and extent of rumen microbial fermentation that are usually associated with digestive disorders such as rumen acidosis (Nagaraja and Titgemeyer, 2007). If an excessive degradation rate of starch is promoted, the risk of ruminal acidosis may increase (Svihus *et al*. 2005), and thus the impact on rumen environment is associated with the nature of dietary starch. Van Barneveld (1999) reported that the fermentation of cereal grains depends on the differences in starch structure from one plant species to another. Despite the amount of starch is higher with corn than barley, the rate of starch fermentation is higher with the latter (Sauvant and Michalet-Doreau, 1988). On the other hand, Calsamiglia *et al*. (2012) proposed that acidosis may be caused by the combined effects of pH and changes in the microbial profile related to the type of diet. The risk of acidosis can be minimized giving adequate amounts of structural carbohydrates, which may avoid a ruminal overload of volatile fatty acids (VFA) and lactate, increasing at the time chewing activity and the flow of salivary buffers. Sauvant *et al*. (1999) conclude that, to avoid the risk of acidosis, diets should contain no more than 25% starch and about 30-40% neutral detergent fibre (NDF) on DM basis. In this regard, several agro-industrial by-products, such as sugarbeet pulp which have a considerable proportion of easily-fermentable hemicelluloses and pectin, are used in ruminants nutrition, rendering high amount of energy when fermented in the rumen (Nocek and Tamminga, 1991). With them, the rumen pH can be maintained because of its own buffering capacity. However, the objective of intensive feedlots is to achieve maximum intake and efficiency of use of energy preserving at the same time a healthy rumen environment.

This work aims to assess the synergistic and antagonistic effects of three mixtures of carbohydrate sources such as maize and barley grain, as sources of starch, and sugarbeet pulp as source of highly fermentable fibre, on *in vitro* fermentation parameters.

II – Material and methods

Three carbohydrate feeds were studied as substrates of incubation, two cereals (barley, var. Gustav and maize grain Dekalb 6667YG), and one by-product (sugarbeet pulp). Substrates were milled through a sieve of 1 mm and used as components of three mixtures, 1:1 maize:barley (MB) and 1:1 and 3:1 maize:sugarbeet pulp (MP and 3MP). Two sets of incubation series were arranged, according to the nature of the inoculum (forage inoculum, FI, and concentrated inoculum, CI), to compare *in vitro* fermentation of substrate mixtures. The rumen fluid was obtained from six lambs weaned abruptly at 7 weeks ± 8 days and fed *ad libitum* afterwards in groups of three with alfalfa hay (FI) for 45 days or with concentrate and barley straw (CI) for 35 days. Then, animals were slaughtered, their rumen contents were individually filtered through a cheesecloth and dispensed into tubes that were frozen in nitrogen liquid and stored at -80 ºC (Prates *et al*., 2010). Before incubation, frozen inocula were thawed in a 39 ºC water bath for 1-2 min. For each inoculum, 3 *in vitro* incubation series of 24 h were carried out, with two bottles per mixture in each series. The fermentation kinetics of experimental feeds were determined by the *in vitro* incubation system of Fondevila and Pérez-Espés (2008), modified by Prates *et al*. (2010). Sealed nylon bags (45um pore size) containing 800 mg of substrates mixtures were incubated in each 125 ml Erlenmeyer flasks, that were filled with 80 ml of incubation solution consisting of 0.20 rumen inoculum and 0.80 of an incubation mixture made up with a macromineral solution, a reduction solution and a buffer solution in which the concentration of bicarbonate ions was buffered to get a pH 5.5 from 0 to 6 h incubation, and a pH 6.5 from 8 to 24 h incubation (Amanzougarene and Fondevila, 2017). The incubation solution was prepared under a CO2 atmosphere, and bottles were maintained at 39 ºC in a water bath throughout the incubation. The pressure of gas produced in each bottle was recorded at 2, 4, 6, 8, 10, 12, 16, 20, and 24 h, and gas volume was expressed per unit of incubated organic matter (OM). Immediately after each gas measurement, fixed volumes of incubation media were extracted by suction

through the filter port, and the exact volume was replaced with incubation solution without rumen inoculum that was maintained anaerobically at 39 ºC. The rate of the liquid phase turnover was adjusted to approximately 6.5ml/h, by replacing liquid media with incubation solution, according to this schedule: 13 ml every 2 h from 0 to 12 h of incubation, and 26 ml every 4 h from 12 to 24 h. Extracted media was used for pH measurement, and then stored for other purposes.

Results for each inoculum were analysed separately by ANOVA using the Statistix 10 software package (Analytical Software, 2010). Each bottle was considered as the experimental unit and the series as a block. The differences were considered significant when $P<0.05$, and a trend for significance was considered when $0.05£P<0.10$. The Tukey test ($P<0.05$) was used for the multiple comparison between means.

III – Results and discussion

1. Pattern of incubation pH

At the start of incubation series, the mean inoculum pH was 6.44 ± 0.12 and 7.01 ± 0.20 for CI and FI, respectively (n=3). When CI was used (Fig. 1a), no differences among mixtures were recorded from 0 to 4h ($P>0.05$), but at 6h the minimum pH was reached, that was highest with MP ($P<0.05$) than MB and 3MP. From 8h, the medium pH increased ($P<0.01$) until reaching the maximum at 20h (6.78, 6.67, 6.67, respectively), maintaining the same treatment differences, and then pH tended to fell ($P=0.081$) at 24h, but no differences between mixtures were recorded ($P>0.05$). When mixtures were incubated with FI (Fig. 1b), no pH differences were recorded among mixtures in the first 4h ($P>0.05$), but at 6h it was lower with MB than with 3MP ($P<0.05$). From 8h onwards, the pH recorded with MP and 3MP was higher than MB ($P<0.05$), not existing differences among the treatments including sugarbeet pulp. For all treatments, the minimum incubation pH was reached after 8h, and then pH for all substrates increased from 10 to 20h, maintaining the lowest values with MB ($P<0.05$), and dropping again at 24h.

Buffer concentration in the incubation media was adjusted to allow for a drop of pH during the first 6h, thus allowing for expression of acidification properties of substrate mixtures in poorly-buffered conditions; afterwards, buffer concentration was increased to simulate pH increase during the low intake daily period in intensive fed ruminants. Such conditions were supported by the minimum medium pH reached at 6 and 8h for CI and FI, respectively, and the maximum pH reached at 20h incubation. However, the extent of pH drop was apparently higher with CI than FI, suggesting that the magnitude of fermentation was higher with CI, as it has been recently demonstrated (Broudiscou *et al*., 2014). When comparing substrate mixtures, the treatment with barley (MB) showed lower incubation pH than those including sugarbeet pulp (MP and 3MP), mainly with CI, because of the higher acidification capacity of barley than maize and the higher acidification capacity of cereal grains than fibrous sources. In fact, the high proportion of starch in 3MP rendered a more acid environment compared with MP considering that the buffering capacity did not greatly differ between maize and sugarbeet pulp (Amanzougarene *et al*., 2017). It is assumed that inclusion of high levels of cereal based concentrates decreases rumen pH (Fondevila *et al*., 1994; Carro *et al*., 2000), as well as the self-buffering capacity of sugarbeet pulp can be expected.

2. *In vitro* gas production

During the whole incubation period, differences ($P<0.001$) in the *in vitro* gas production between the different mixtures were recorded with CI (Fig. 2a). The volume of gas recorded was highest with MB at 2h and from 6 to 16h ($P<0.05$), whereas it was lowest with 3MP from 2 to 8h and with MP from 20h onwards ($P<0.05$). No differences ($P>0.05$) were recorded between MP and 3MP from

10 to 16h, nor between MB and 3MP at 20 and 24h. With FI (Fig. 2b), differences between substrate mixtures on gas production were recorded throughout all the incubation period (P<0.05), the volume of gas recorded by 3MP being lowest (P<0.05) up to 4h, and lower with 3MP than with MB from 6h onwards (P<0.05), whereas differences between MB and MP were only recorded after 24h.

Although not contrasted statistically, the extent of gas production was greater with CI than FI, as it can be expected from results in medium pH. This can be explained by the fact that microorganism of the former inoculum should be better adapted than those from a fibrous diet for the fermentation of this kind of substrates (Amanzougarene *et al*., 2017). Among mixtures, MB recorded a higher volume of gas production than MP and 3MP in both inocula. Comparison between MB and 3MP in CI is determined by the faster rate of fermentation of barley respect to maize, which can be due to the floury starch of barley (Chevalier, 2001), effect that is balanced at later stages of fermentation (at 20 and 24h). However, the lower gas production in 3MP when FI was used can be attributed to the low capacity of microbiota induced by a forage diet for fermenting maize vitreous starch (Amanzougarene *et al*., 2017). Differences in gas production comparing MP to MB and 3MP were of minor magnitude, and even disappear at later stages of incubation mainly with FI probably due to scarce differences between utilisation of slowly fermentable maize starch and rapidly fibre fermentable of sugarbeet pulp.

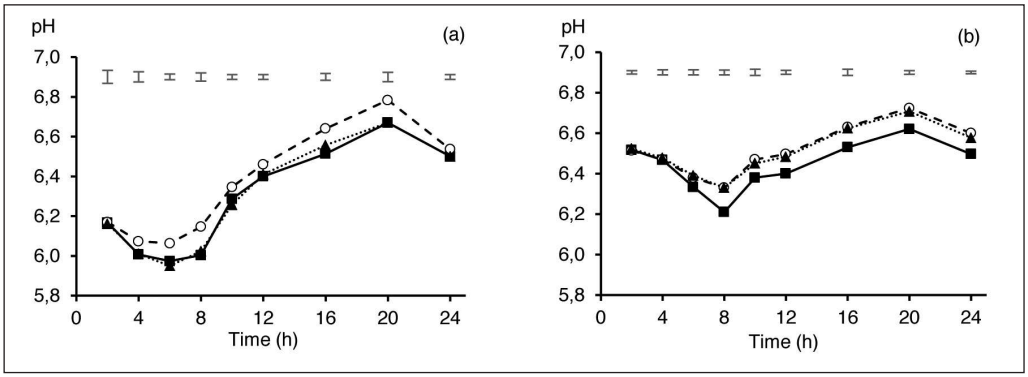

Fig. 1. Pattern of incubation pH from MB; ■, MP; ○, and 3MP; ▲, when incubated *in vitro* with inoculum from a concentrate diet (CI, Fig. 1a) or from a forage diet (FI, Fig. 1b). Upper bars show standard error of means.

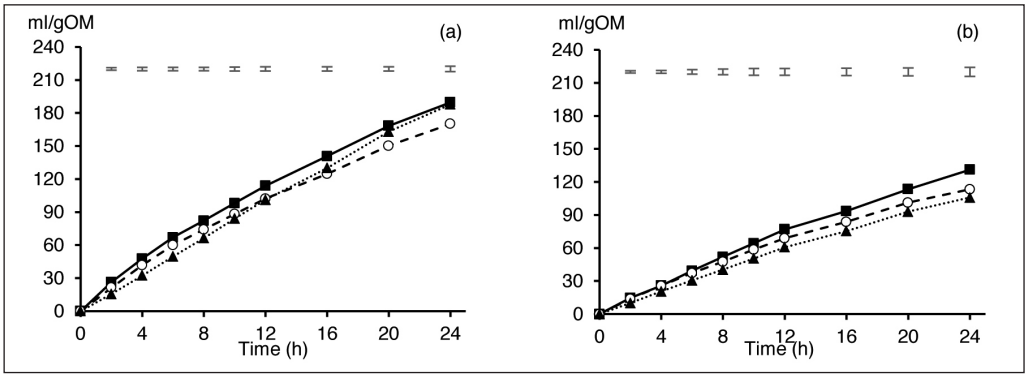

Fig. 2. Pattern of incubation pH from MB; ■, MP; ○, and 3MP; ▲, when incubated *in vitro* with inoculum from a concentrate diet (CI, Fig. 2a) or from a forage diet (FI, Fig. 2b). Upper bars show standard error of means.

IV – Conclusions

In both concentrate and forage environments, inclusion of 50% fibrous source (MP) maintained a more stable pH pattern whereas fermentation was not noticeably depressed compared to higher starch proportion mixtures like 3MP (75 % cereal; 25% sugarbeet pulp), or even a high starch substrate such as MB (100% cereal). From the results obtained, it can be concluded that combine cereal with by-products, as sugarbeet pulp, can promote positive effects on ruminal fermentation due to synergic or antagonist interactions between diet components.

Acknowledgements

This work was supported by the Spanish Government through the MINECO project AGL 2013-46820, with the participation of the Department of Industry and Innovation of the Government of Aragón, and the European Social Fund. S. Yuste was supported by a FPU fellowship from the Ministerio de Educación, Cultura y Deporte (Government of Spain). There were no conflicts of interests.

References

Amanzougarene Z., Yuste S., de Vega A. and Fondevila M., 2017. *In vitro* fermentation pattern and acidification potential of different sources of carbohydrates by ruminants giving high concentrate diets, *Spanish Journal of Agricultural Research*, Vol. 15 (2), e0602. http://dx.doi.org/10.5424/sjar/2017152-10753.

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(9), p. 1751-1757.

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

Broudiscou L.P., Offner A. and Sauvant D., 2014. Effects of inoculum source, pH, redox potential and headspace di-hydrogen on rumen *in vitro* fermentation yields, *Animal*, 8, p. 931-937.

Calsamiglia S., Blanch M., Ferret A. and Moya D., 2012. Is subacute ruminal acidosis a pH related problem? Causes and tools for its control, *Anim. Feed Sci. Technol.,* 172, p. 42-50.

Carro M.D., Valdés C., Ranilla M.J. and Gonzáles J.S., 2000. Effect of forage to concentrate ratio in the diet on ruminal fermentation and digesta flow kinetics in sheep offered food at a fixed and restricted level of intake, *Anim. Sci.,* 70, p. 127-134.

Chevalier F.H., 2001. La digestion ruminale des amidons : cinétique et applications. Thèse d'exercice, Université Paul Sabatier – Toulouse III, 2001, 115 p.

Fondevila M. and Perez-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, *Anim. Feed Sci. Technol.,* 144, p. 196-211.

Fondevila M., Castrillo C., Guada J.A. and Barcells J., 1994. Effect of ammonia treatment and carbohydrate supplementation of barley straw on rumen liquid characteristics and substrate degradation by sheep, *Anim. Feed Sci. Technol.,* 50, p. 137-155.

Nagaraja T.G. and Titgemeyer E.C., 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook, *J. Dairy Sci.,* 90: E17-E38.

Nocek J.E. and Tamminga S., 1991. Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition, *J. Dairy Sci.,* 74, p. 3598-3629.

Prates A., de Oliveira J.A., Abecia L. and Fondevila M., 2010. Effects of preservation procedures of rumen inoculum on *in vitro* microbial diversity and fermentation, *Anim. Feed Sci. Technol.,* 155, p. 186-193.

Sauvant D. and Michalet-Doreau B., 1988. Les aliments concentrés. In: *Alimentation des bovins, ovins, caprins.* Chap 17. INRA, p. 337-349.

Sauvant D., Meschy F. and Mertens D., 1999. Les composants de l'acidose ruminale et les effets acidogènes de l'acidose, *INRA Prod. Anim.,* 12, p. 49-60.

Svihus S.B., Uhlen A.K. and Harstad O.M., 2005. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: a review, *Anim. Feed Sci. Technol.,* 122, p. 303-320.

Van Barneveld S.L., 1999. Chemical and physical characteristics of grains related to variability in energy and amino acid availability in ruminants: A review, *Aust. J. Agric. Res.,* 50, p. 651-666.