Effects of a yeast enzymatic hydrolyzate on in vitro ruminal fermentation

Díaz A., Saro C., Tejido M.L., Sosa A., Martínez M.E., Galindo J., Carro M.D., Ranilla M.J.

in

Ranilla M.J. (ed.), Carro M.D. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.).
Challenging strategies to promote the sheep and goat sector in the current global context

Zaragoza : CIHEAM / CSIC / Universidad de León / FAO
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 99

2011
pages 181-186

Effects of a yeast enzymatic hydrolyzate on *in vitro* ruminal fermentation

A. Díaz*,, C. Saro**,***, M.L. Tejido**,***, A. Sosa*,****, M.E. Martínez**, J. Galindo****, M.D. Carro**,*** and M.J. Ranilla**,***

*Universidad de Matanzas "Camilo Cienfuegos", Matanzas (Cuba)
**Departamento de Producción Animal. Universidad de León, 24071 León (Spain)
***Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n. 24346 Grulleros, León (Spain)
****Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, La Habana (Cuba)

Abstract. The incessant search of new feed additives for ruminants is one of the main objectives of nutritionists. In this sense a great number of compounds that act at ruminal level and improve forage utilization, have been studied. The objective of this study was to determine the effect of a yeast enzymatic hydrolyzate of *Saccharomyces cerevisiae* and its different fractions on ruminal fermentation under *in vitro* conditions. Forage of *Pennisetum purpureum* vc Cuba CT-115 was used as substrate for *in vitro* incubations. Four doses (0, 50, 100 and 200 µL/50 ml incubation medium) of total hydrolyzate and two fractions of it (supernatant and gross pellet) were investigated. In all cases, best results were obtained with the higher dose (200 µL). With all fractions, this dose increased (P<0.05) total volatil fatty acids and propionate production. Also an increase on butyrate production was observed with supernatant fraction and total hydrolyzate (P<0.05). Isoacids production was increased (P<0.05) only with the supernatant fraction. Gas production was stimulated (P<0.05) by the dose 200 µL of the pellet fraction and by the doses 50 and 200 µL of total hydrolyzate. The supernatant fraction did not affect (P<0.05) the gas production. Ammonia-N concentration increased (P<0.05) with 200 µL of supernatant fraction and total hydrolyzate. Final pH decreased (P<0.05) with doses 100 and 200 µL of all fractions. Dry matter disappearance, neutral detergent fibre disappearance and true dry matter degradability were not affected (P<0.05) by any fraction or dose. We conclude that the use of this enzymatic hydrolyzate and their different fractions are able to stimulate the *in vitro* ruminal fermentation of *Pennisetum purpureum* vc Cuba CT-115.

Keywords. Feed additives – *Saccharomyces cerevisiae* – Forage diet.

Effets d'un hydrolysat de levure sur la fermentation ruminale in vitro

Résumé. *La recherche incessante de nouveaux additifs pour l'alimentation des ruminants est l'un des objectifs principaux des nutritionnistes. Dans ce sens, un grand nombre de composés qui agissent au niveau ruminal et améliorent l'utilisation de fourrage ont été étudiés. L'objectif de cette étude a été de déterminer l'effet d'un hydrolysat enzymatique de la levure* Saccharomyces cerevisiae *et de ses différentes fractions sur la fermentation ruminale dans des conditions in vitro. Le fourrage de* Pennisetum purpureum *vc Cuba CT-115 a été employé comme substrat pour des incubations in vitro. Quatre doses (0, 50, 100 et 200 milieu d'incubation de µL/50 ml) de l' hydrolysat total et deux fractions (pellet brut et surnageant) ont été étudiées. Dans tous les cas, les meilleurs résultats ont été obtenus avec la dose plus élevée (200 µL). Avec toutes les fractions, cette dose a augmenté (P<0.05) la production totale d'acides gras volatiles et de propionate. Aussi, avec la fraction surnageant et l'hydrolysat total, la production de butyrate a augmenté (P<0.05). La production de gaz a été stimulé (P<0.05) avec la dose 200 µL du pellet et avec les doses 50 and 200 µL de l'hydrolysat total. La fraction surnageant n'a pas affecté (P<0.05) la production totale de gaz. La concentration d'azote ammoniacal a augmenté (P<0.05) avec 200 µL du surnageant et d'hydrolysat total. Le pH final a diminué (P<0.05) avec les doses 100 and 200 µL de toutes les fractions. La dispparition de MS, de la NDF et la digestibilité réelle de la MS n'ont pas été affectés (P<0.05) par aucune fraction ou dose. On conclut que l'usage de cet hydrolysat enzymatique and leurs fractions ont été capables de stimuler la fermentation ruminale in vitro de* Pennisetum purpureum *vc Cuba CT-115.*

Mots-clés. *Additives* – Saccharomyces cerevisiae – *Fourrage.*

I – Introduction

Microbial feed additives for ruminants are used for different purposes, but with the final aim of enhancing productivity by modifying digestion and metabolism and leading to the improvement of milk and/or meat production. In adult animals, they are intended to increase propionate and decrease methane and lactate production and deamination by modifying rumen microbial populations (Galindo and Marrero, 2005). All these changes are intended to improve energy and nitrogen metabolism in the rumen (Carro and Ranilla 2002).

A number of strategies have been used to enhance ruminal fermentation. Biological additives have included microorganisms, enzymes and plant products (Jouany, 1994; Dean *et al*., 2005; Busquet *et al*., 2006; Carro *et al*., 2006). Direct-fed microbials offer a great potential for manipulation of ruminal fermentation and the yeast *Saccharomyces cerevisiae* is an especially attractive organism. The objective of this study was to determine the effect of a yeast enzymatic hydrolyzate of *S. cerevisiae* and its different fractions on ruminal fermentation under *in vitro* conditions using batch cultures of mixed rumen micro-organisms.

II – Materials and methods

1. Substrate and additives

A tropical forage, *Pennisetum purpureum* cv. CT-115, was used as substrate for the batch incubations. Samples were ground at 1 mm particle size. The substrate contained 86.55% organic matter, 5.01% crude protein, 71.16% neutral detergent fibre (aNDF), 38.96% acid detergent fibre (aADF) and 5.42% lignin.

Four fractions of a *S. cerevisiae* hydrolyzate were investigated: total hydrolyzate, supernatant, gross pellet and washed pellet. To obtain the last three fractions the quantity corresponding to each dose it centrifuged at 19000 x *g*, during 15 minutes at 4ºC. The treatments consisted of four doses of each fraction: 0, 50, 100 and 200 µl/50 ml incubation medium.

2. *In vitro* fermentation of substrates

Rumen fluid was obtained from 4 rumen-cannulated merino sheep fed a 70:30 alfalfa hay:concentrate diet, distributed in two equal portions at 09:00 and 18:00 h. Sheep were managed according to protocols approved by the León University Institutional Animal Care and Use Committee. Rumen contents were obtained immediately before the morning feeding from each sheep, mixed and strained through 2 layers of cheesecloth into stoppered flasks and transported to the laboratory within 30 min. Particle-free fluid was filtered through nylon bag of 100mm pore size and mixed with the buffer solution of Goering and Van Soest (1970; no trypticase added) in a proportion 1:4 (vol/vol) at 39ºC under continuous flushing with CO_2. Fifty ml of buffered rumen fluid were added into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39ºC.

Samples of substrate (500 mg) were weighed into 120 ml serum bottles. Additives were applied inside the bottles immediately before adding buffered rumen fluid. Four identical incubation runs were carried out, each of them with the mixed rumen fluid of the four sheep.

All bottles were withdrawn 24 h after inoculation. At the end of the incubation period, total gas production was measured in each bottle using a pressure transducer and a calibrated syringe.

Bottles were uncapped, the pH was measured immediately with a pH meter, and the fermentation was stopped by swirling the bottles in ice. One milliliter of content was added to 1 ml of de-

proteinising solution (i.e. metaphosphoric acid (100 g/l) and crotonic acid (0.6 g/l)) for volatile fatty acid (VFA) determination and 2ml were added to 2ml 0.5M HCl for ammonia-N analysis. Finally, contents of the bottles were transferred to previously weighed filter crucibles.

Crucibles were dried at 50ºC, weighed and the residue was analysed for aNDF to calculate true DM degradability (TDMD; Van Soest *et al*., 1991) and aNDF degradability (aNDFD).

3. Analytical procedures

Dry matter (ID 934.01), ash (ID 942.05) and N (ID 984.13) were determined according to the Association of Official Analytical Chemists (1999). Fibre analyses were carried out according to Van Soest et al. (1991) using an ANKOM220 Fibre Analyzer unit (ANKOM Technology Corporation, Fairport, NY, USA). Concentrations of VFA and ammonia-N in rumen fluid were determined as described by Carro and Miller (1999).

4. Calculations and statistical analyses

Data were analyzed using the PROC MIXED procedure of SAS (2002). Four concentrations of additive (i.e. 0, 0.5, 5, 10 mg/L) and the interaction of additive×fraction were included in the model as a fixed effect, whereas incubation day was considered as a random effect. Significance was declared at $P<0.05$, whereas $P<0.10$ values were considered to be a trend. Within each additive treatment and inoculum, there were 4 values for each of the measured variables.

III – Results and discussion

The effects of the hydrolyzate and its different fractions on gas production parameters are shown in Table 1. The only parameter affected by fraction was gas production ($P<0.001$),and therefore, only significance P values are shown in the Table. Adding the hydrolyzate and its fractions to the *in vitro* cultures linearly increased ($P=0.001$) gas production, suggesting a improved substrate fermentation compared to the control.

Final pH decreased linearly ($P=0,001$) as concentrations of additives increased, whereas NH_3-N concentrations linearly increased ($P=0.049$) for the three fractions studied. Sttater and Slyter (1974) reported that the concentration at which ammonia nitrogen became limiting for a rumen microbial population continuously cultured in an artificial rumen and maintained in steady state condition was 50 mg NH_3/l of ruminal fluid. In this sense, our data are all above this value, and also above the recommendations suggested by Leng (1991), who proposed a minimum value of 200 mg N-NH_3/l. Although the substrate incubated had a very low protein content (about 5%), the in vitro culture media includes N, and therefore, it could have balanced the shortage of N by providing non-protein N (Álvarez *et al*., 1976; Boniface *et al*., 1986; Valdés and Castillo, 1993) and the available ammonia to the microbial populations (Silva *et al*., 1989; Leng, 1990). Also, the N supplied by the hydrolyzate and its fractions has probably, compensated the protein deficiency of substrate, stimulating its fermentation by this mechanism.

There was no significant effect ($P<0.05$) of the different hydrolyzate fractions on VFA production, whereas VFA production increased linearly ($P<0.05$) as doses of additives increased (15%, 16% and 19% for 200 µl dose compared to the control, for gross pellet, supernatant and total hydrolyzate, respectively). Production of acetate, propionate and butyrate also increased ($P<0.05$) linearly with increasing doses of gross pellet, supernatant and total hydrolyzate, but there was no effect ($P>0.05$) on others VFA production (calculated as the sum of isobutyrate, isovalerate and valerate). Adding the hydrolyzate and its fractions did not affect ($P>0.05$) the acetate:propionate ratio, which was probably related to the proporcional increase in the production both acids.

Carro, M.D. and Ranilla, M.J., 2002. Los aditivos antibióticos promotores del crecimiento de los animales: Situación actual y posibles alternativas. Nutrición. Albéitar 56: 46-49.

Carro, M.D. and Miller, E.L., 1999. Effect of supplementing a fibre basal diet with different nitrogen forms on ruminal fermentation and microbial growth in an in vitro semicontinuous culture system (Rusitec). Br. J. Nutr. 82: 149-157.

Carro, M.D., Ranilla, M.J., Giráldez, F.J. and Mantecón, A.R., 2006. Effects of malate on diet digestibility, microbial protein synthesis, plasma metabolites, and performance of growing lambs fed a high-concentrate diet. J. Anim. Sci. 84:405-410.

Chademana, I. and Offer, N.W., 1990. The effect of dietary inclusion of yeast culture on digestion in the sheep. Anim. Prod.; 50: 483-489.

Dean, D.B., Adesogan, A.T., Krueger, N. and R. Littell, C., 2005. Effect of fibrolytic enzymes on the fermentation characteristics, aerobic stability, and digestibility of bermudagrass silage. J. Dairy Sci. 88:994-1003.

Díaz Reyes, A., 2008. Efecto de un hidrolizado enzimático de levadura Saccharomyces cerevisiae en la fermentación ruminal in vitro de dietas fibrosas. Tesis presentada en opción al Título Académico de Master en Producción Animal para la zona tropical. Instituto de Ciencia Animal. La Habana. Cuba.

Galindo Blanco, J. and Marrero, Y., 2005. Modificación de la fermentación microbiana ruminal. Rev. Cubana. Ciencias Agrícolas. 39.:439-450.

Goering, H.K. and Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook no. 379. Agriculture Research Service, United States Department of Agriculture. Washington, DC, USA.

Jouany, J.P., 1994. Methods of manipulating the microbial metabolism in the rumen. Ann Zootech. 43:49-62.

Leng, R.A., 1990. Factors affecting the utilization of "poor quality" forages by ruminant animals particularly under tropical conditions. Nutritional Research Reviews 3: 277-303.

Leng, R.A., 1991. Application of biotechnology to nutrition of animals in developing countries. FAO Animal Production and Health paper 90. Roma, Italia: 146pp.

Lila, Z.A., Mohammed, N., Yasui, T., Kurokawa, Y., Kanda, S. and Itabashi, H., 2004. Effects of Saccharomyces cerevisiae twin of live cells on mixed ruminal microorganism fermentation in vitro. J. Dairy Sci. 82, 1847-1854.

Marrero Rodríguez, Y., 2005. Las levaduras como mejoradoras de la fermentación ruminal de dietas con alto contenido de fibra. Tesis presentada en opción al grado científico de Doctor en Ciencias Veterinarias. Instituto de Ciencia Animal. La Habana. Cuba.

Miller-Webster, T., Hoover, W., Holt, M. and Nocek, J.E., 2002. Influence of yeast culture on ruminal microbial metabolism in continouos culture. J.Dairy Sci. 85, 2009-2014.

Mutsvangwa, T., Edwards, I.E., Topps, J.H. and Paterson, G.F.M., 1992. The effect of dietary inclusion of yeast culture (Yea-Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls. Anim. Prod. 55, 35-41.

Newbold, C.J., 1990. Probiotics as feed additives in ruminant diets. 51st Minnesota Nutrition Conference. p 102-118.

Roa, M.L., Bárcena-Gama, J.R., González, M.S., Mendoza, G.M., Ortega, M.E. and García, C.B., 1997. Effect of fiber source and yeast culture (Sc1026) on digestion and the enviroment in the rumen of cattle. Anim. Feed Sci. Technol.; 64: 327-336.

Silva, A.T., Greenhalgh, J.F.D. and Orskov, E.R., 1989. Influence of ammonia treatments and supplementation on the intake, digestibility and weigth gain of of sheep and cattle on barley staw diets. Anim. Production 48: 99-108.

Sttater, L.D. and Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein in vitro. Br. J. Nutr. 32: 199-208.

Valdés, G. and Castillo, F., 1993. Sistema de manejo de machos bovinos en pastoreo I. suplementación con miel y un alto nivel de urea. Rev. Cubana Ciencias Agrícolas. 27: 160-163.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci.,74(10), 3583-3597.