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In vitro fermentation of diets including agroindustrial by-products in batch cultures of ruminal microorganisms from goats

M. Romero-Huelva[1], M.D. Carro[2] and E. Molina-Alcaide[3]

[1]UNIPROCA, calle Carnicerías, 17, 45600, Talavera de la Reina, Spain
[2]Dpto. de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid
Ciudad Universitaria, 28040 Madrid. Spain
[3]Estación Experimental del Zaidín (CSIC), Profesor Albareda, 1, 18008 Granada, Spain

Abstract. In the last years, there has been an increasing interest in the use of alternative feeds, such as agroindustrial by-products, in ruminants feeding. The aim of the present work was to study the effects of including variable amounts of different by-products (tomato fruits, citrus pulp, brewer´s grains and brewer´s yeast), previously processed and dried, in the concentrate of dairy goats on *in vitro* ruminal fermentation. Six concentrates were formulated and mixed with alfalfa hay in 1:1 proportion before being fermented *in vitro* using batch cultures of mixed rumen microorganisms from goats. The kinetics of gas production was evaluated in 72-h incubations and the fermentation variables were determined after 24 h incubation. Gas production data were adjusted to the exponential model $y = A [1 – e^{(-c•t)}]$. There were no differences among diets either on pH (P = 0.091) or molar proportions (P = 0.613 to 0.999) of individual volatile fatty acids (VFA), but total VFA production was greater (P < 0.005) and the amount of CH_4 was lower (P < 0.001) in five of the diets including by-products compared with the control diet. The inclusion of by-products resulted in greater values of gas production and gas production rate (P < 0.004 and 0.001, respectively) in four of the diets. The results of this study indicate that by-products from tomato and citrus juice and beer industry could be included in ruminant's diets, promoting better ruminal fermentation pattern and reducing the environmental impact of animal production. The potential of agro-industrial by-products to substitute conventional ingredients in small ruminant diets should be further investigated.

Keywords. Batch Cultures – Concentrate – By-products – Methane Production – Ruminal Fermentation.

Fermentation* in vitro *de régimes comprenant des sous-produits agroindustriels dans des cultures de microorganismes du rumen de chèvres

Résumé *- Au cours des dernières années on a constaté intérêt croissant pour l'utilisation d'aliments alternatifs tels que les sous-produits agro-industriels, dans l'alimentation des ruminants. Le but du présent travail était d'étudier les effets de l'inclusion d'un mélange de sous-produits (fruits de tomate, pulpe d'agrumes, grains et levure de bière), préalablement transformés et séchés dans des fours solaires à convection, dans le concentré des chèvres laitières sur la fermentation ruminale* in vitro. *Six concentrés ont été formulés et mélangés avec du foin de luzerne en proportion 1: 1 avant d'être fermentés in vitro en utilisant des cultures de micro-organismes provenant du rumen de chèvres. La cinétique de la production de gaz a été évaluée sur la base d'incubations de 72 heures et les variables de fermentation ont été déterminées après 24 h d'incubation. Les données de production de gaz ont été ajustées au modèle exponentiel suivant:* $y = A [1 – e^{(-c • t)}]$. *Il n'y avait pas de différence entre les régimes ni sur le pH (P = 0.091) ni sur les proportions molaires (P = 0,613 - 0,999) des acides gras volatils individuels (VFA), mais la production totale d'acides gras voltails (AGV) était plus élevée (P < 0,005) et la quantité de* CH_4 *était plus faible (P < 0,001) dans cinq des régimes incluant les sous-produits par rapport au régime témoin. L'inclusion de sous-produits a entraîné des valeurs plus élevées de la production de gaz (P = 0,004) et des taux de production de gaz (P = 0,001) dans quatre des régimes alimentaires. Les résultats de cette étude indiquent que les sous-produits de l'industrie de la tomate, du jus d'agrumes et de la bière pourraient être inclus dans les régimes alimentaires des ruminants, en ameliorant la fermentation du rumen et en reduisant l'impact environnemental de la production animale. Le potentiel des sous-produits agro-industriels de substituer les ingrédients classiques dans les régimes de petits ruminants devrait être étudié plus en profondeur.*

Mots-clés. *Cultures de Micororganismes Ruminales – Concentré – Sous-produits – Production de Méthane – Fermentation Ruminale.*

I – Introduction

Goat production systems are increasingly changing to intensive models, in which feeding is based on concentrates mainly composed of cereals and other expensive ingredients. The use of agroindustrial by-products in goats feeding could reduce production cost (Ben-Salem and Znaidi, 2008) without altering yield and animal products quality, and some by-products may decrease methane emissions (Molina-Alcaide *et al*., 2010; Romero-Huelva *et al*., 2012). However, a wide range of by-products is available and nutritive evaluation is necessary prior to their use in goats feeding. The aim of the present work was to study the effects of including variable amounts of different by-products (tomato fruits, citrus pulp, brewer´s grain and brewer´s yeast) in the concentrate of diets formulated for dairy goats on their *in vitro* ruminal fermentation.

II – Materials and methods

1. By-products, experimental diets and animals

All by-products were provided by the company Aspero S.A. (Sevilla, Spain). Tomato fruits were cut and dried at 65ºC in a convection oven under controlled conditions. The other by-products were citrus pulp, brewer´s grain and brewer´s yeast and were also dried at 65ºC. Seven experimental diets were formulated based on alfalfa hay and concentrate in a 50:50 ratio. The control concentrate was a commercial one and was composed of corn, wheat bran, sunflower meal, soya hulls, sorghum, soya meal, wheat, calcium carbonate, sugar beet molasses, sepiolite, palm soap, sodium chloride and vitamin-mineral mixture in proportions of 250, 210, 120, 100, 100, 108, 52, 10, 10, 10, 12, 6 and 12 g per kg of concentrate (fresh matter basis). Six experimental concentrates were formulated by replacing 62.5 g of corn with 62.5 g of tomato fruits (T1); 94 g of corn with 94 g of tomato fruits (T2), 125 g of corn with 125 g of tomato fruits (T3); 125 g of corn, 25 g of wheat bran and 25 g of sunflower meal with 125 g of tomato fruits and 50 g of citrus pulp (T3C); 125 g of corn, 25 g of wheat bran and 60 g of sunflower meal with 125 g of tomato fruits, 50 g citrus pulp and 35 g of brewer’s grains (T3CB); 125 g of corn, 25 g of wheat bran, 60 g of sunflower meal and 25 g of soya flour with 125 g of tomato fruits, 50 g of citrus pulp, 35 g of brewer’s grains and 25 g of brewer’s yeast (T3CBY). Chemical composition of experimental diets is given in Table 1. Diets ingredients (alfalfa hay and concentrates) were ground (1 mm) before incubation in batch cultures.

Table 1. Chemical composition (g/kg dry matter, unless other stated) and estimated price (€/ton) of the experimental diets

	Diets						
	Control	T1	T2	T3	T3C	T3CB	T3CBY
Dry matter, g/kg fresh	925	913	912	908	914	903	911
Organic matter	860	835	835	831	836	822	839
Crude protein	176	174	181	174	163	180	168
Neutral detergent fibre	404	422	426	423	410	394	416
Acid detergent fibre	252	254	262	263	262	251	260
Acid detergent lignin	46.9	49.6	50.2	50.8	48.0	47.5	47.7
Ether extract	23.2	23.6	23.4	23.1	23.2	25.9	26.0
Gross energy, MJ/kg dry matter	154	154	154	153	152	153	156
Price, €/ton	219	211	208	204	199	198	194

Three adult dry non-pregnant rumen-fistulated Murciano-Granadina goats fed alfalfa hay at maintenance energy requirements were used as rumen contents’ donors for the *in vitro* incubations. Animals were cared and handled in accordance with the Spanish guidelines for experimental animal protection (Royal Decree 53/2013) in line with the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (2012/707/UE). All procedures were approved by the Ethic Committee for Animal Experimentation of the Spanish Research Council and the Junta de Andalucía (approval numbers 24/05/2016/091 and 22/06/2016/115, respectively).

2. *In vitro* incubations

Three 72-h *in vitro* incubation runs were conducted, and four bottles per diet and four blanks (bottles without substrate) were used in each run. Rumen contents were collected from the three goats before the morning feeding, pooled and immediately taken to the laboratory into thermal flasks. Rumen contents were strained through four layers of cheesecloth and mixed with a buffer solution (Goering and van Soest, 1970) in a 1:4 ratio (vol / vol) at 39°C under continuous flushing with CO_2. Samples (0.5 g) of each substrate were carefully weighed into 120-ml bottles, and 60 ml of the buffered rumen fluid were anaerobically added into each bottle. Bottles were then capped and incubated at 39º C. In two of the four bottles for each diet and two blanks, pressure and gas volume were measured at 2, 4, 6, 8, 12, 24, 48 and 72 h of incubation. In the remaining two bottles for each diet, the gas produced after 24 h and a gas sample (about 5 ml) was taken for analysis of methane. Bottles were then uncapped, the pH was measured immediately *(*Crison Basic 20 pH-meter*,* Crisson Instruments, Barcelona. Spain) and 2 ml of bottles contents were added to 2 ml of deproteinising solution (20 g of metaphosphoric acid and 0.6 g of crotonic acid per litre) for volatile fatty acid (VFA) determination. Chemical composition of diets was determined following the procedures of the AOAC (2005). Analysis of methane and VFA were carried out by gas chromatography as described by Molina-Alcaide *et al*. (2017).

3. Calculations and statistical analyses

The gas produced in batch cultures was adjusted to the exponential model: $y = A (1 - e^{(-c * t)})$, where A is the asymptotic gas production (ml), *c* is the fractional rate of gas production (h^{-1}), and t is the time of gas measurement (h). The average gas production rate (AGPR; mL gas/h) is defined as the average gas production rate between the start of the incubation and was calculated as $AGPR = A c / [2 (\ln 2 + c)]$. Data were analysed by ANOVA considering the diet as a fixed effect and incubation run as a random effect. When a significant effect of diet was found, post hoc comparison of means was made using the Tukey test. Differences were considered significant at $P < 0.05$, and $P < 0.10$ values were declared as trend and discussed. The SPSS for Windows (version 19.0. 2010; SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

III – Results and discussion

The inclusion of by-products in the diets did not show any significant effect ($P = 0.091$) on final pH values (Table 2). There were differences ($P = 0.005$) among diets in VFA production, values being higher for the diets including by-products compared with the control one. On the contrary, both molar proportions of individual VFA and acetate/propionate ratio were not affected ($P = 0.613$ to 0.999) by the incubated diet. Methane production was lower ($P < 0.001$) for diets T3, T3C, T3CB and T3CBY compared with the control diet, but diet T1 showed higher ($P < 0.001$) methane production than the control. Although all diets containing 94 (T2) or 125 g of tomato fruit per kg (T3, T3C, T3CB and T3CBY) reduced significantly methane production compared with the control diet, the lowest values for methane production were observed for the T3C diet, with a 22% reduction. In addition, all diets including tomato fruits, with the exception of T1, reduced the methane/VFA ratios compared with the control, which shows higher fermentation efficiency, as methane represents an energy loss and VFA are the main source of energy and precursors for the host animal. These results would indicate an antimethanogenic effect of tomato fruits and confirm previous results from our group in dairy goats fed diets containing tomato fruits (Romero-Huelva and Molina-Alcaide, 2013). However, the reduction in methane emissions found with diets including by-products were not accompanied by increased propionate proportions, which suggests that other metabolic pathways could have acted as hydrogen sinks. The observed differences among diets in methane production may rely upon differences in their chemical composition, or could be even derived from the presence in the by-products of plant secondary compounds (polyphenols, tannins, saponins, etc.), which could act as natural safe antimethanogenic compounds, alternative to chemical ones, as suggested by Patra and Saxena (2010). These compounds may have an effect on the ruminal microbiota, mainly on archaea and protozoa communities, although it is still unknown which genera or species of archaea are most involved in ruminal methane production (Morgavi *et al*., 2010).

Table 2. Average values of pH, total volatile fatty acids (VFA), molar proportions of individual VFA, acetate to propionate ratio, methane production and methane/VFA ratio after 24 h incubation of concentrates in batch cultures

	Diets								
	Control	**T1**	**T2**	**T3**	**T3C**	**T3CB**	**T3CBY**	**SEM**	**P-value**
pH	6.70	6.71	6.69	6.69	6.68	6.71	6.68	0.002	0.091
Total VFA, mmol	2.51^{a}	2.63^{b}	2.69^{bc}	2.69^{bc}	2.68^{bc}	2.73^{c}	2.75^{c}	0.023	0.005
VFA, mol/100 mol									
Acetate	64.8	65.5	64.3	65.3	66.0	64.8	65.4	0.141	0.822
Propionate	21.5	21.7	22.7	22.2	21.7	22.3	22.2	0.379	0.999
Isobutyrate	1.19	1.12	1.12	1.10	1.05	1.08	1.04	0.019	0.979
Butyrate	9.54	8.96	9.15	8.87	8.68	9.16	8.82	0.245	0.999
Isovalerate	1.63	1.49	1.49	1.41	1.34	1.38	1.27	0.050	0.991
Valerate	1.34	1.27	1.28	1.21	1.18	1.23	1.19	0.012	0.613
Acetate/propionate	3.08	3.05	2.89	2.99	3.09	2.95	3.01	0.057	0.999
Methane, mL/g incubated DM	42.3^{d}	45.7^{e}	35.7^{ab}	37.7^{bc}	33.1^{a}	37.2^{bc}	38.7^{c}	0.633	<0.001
Methane/AGV, mL/mmol	7.80^{b}	7.96^{b}	6.05^{a}	6.37^{a}	5.63^{a}	6.11^{a}	6.37^{a}	0.254	0.017

As shown in Table 3, the inclusion of by-products in the concentrate resulted in higher (P < 0.05) values of asymptotic gas production for all experimental diets compared with the control diet. In addition, T3, T3C, T3CB and T3CBY diets showed greater (P < 0.05) gas production rates than the control, T1 and T2 diets. There were also differences (P < 0.001) among diets in AGPR values, with all diets excepting T2 having greater (P < 0.05) values than the control one. The greater gas production values observed for the diets including by-products are consistent with the increased VFA production detected for these diets in the 24-h incubations. The results suggest that the diets including by-products were more extensively fermented, which might be due to a better supply of nutrients to the ruminal microbiota as a consequence of a better synchronization between nitrogen and energy degradation by ruminal microorganisms (Bach *et al*., 2005). Other factors such as energy and nitrogen quality of the diet could influence nitrogen and energy utilization (NRC, 2001). Moreover, some of these diets promoted better fermentation patterns, as indicated by the reduced methane production and methane/VFA ratios.

The combination of tomato fruits, citrus pulp and either brewer's grains (T3CB concentrate) or brewer's yeast (T3CBY concentrate) resulted in the greatest AGPR values, thus indicating a more rapid fermentation. Moreover, these diets also had the lowest cost (198 and 194 €/ton, respectively), being 9.6 and 11.4% lower than that of the control concentrate. These by-products also allowed higher replacement levels of conventional ingredients and lead to lower methane emissions, which has strong environmental implications in a world with an exponential growing population.

Table 3. Average values of gas production parameters after 72 h incubation of experimental diets in batch cultures

	Diets								
	Control	T1	T2	T3	T3C	T3CB	T3CBY	SEM	P-value
Gas produced, mL	338[a]	343[ab]	346[bc]	350cd	352[d]	364[e]	362[e]	1.364	<0.001
A, ml	118[a]	121[b]	121[b]	121[b]	123[cd]	125[d]	124[d]	0.679	0.004
c, h^{-1}	0.079[a]	0.081[a]	0.087[a]	0.096[b]	0.098[b]	0.102[b]	0.103[b]	0.003	0.001
AGPR (ml / g dry matter)[1]	6.71[a]	7.09[ab]	7.56[b]	8.33[c]	8.69[cd]	9.14[d]	9.23[d]	0.273	<0.001

[1] Average gas production rate, calculated as AGPR = A *c* / [2 (ln 2 + c)].

IV – Conclusions

The replacement of conventional ingredients in a diet for lactating goats with a mixture of tomato fruits, citrus pulp, brewer's grain and brewer's yeast resulted in increased *in vitro* fermentation extension and rates. Moreover, the use of by-products in dairy goats could contribute to minimize the negative environmental impact associated with methane emissions from ruminants and to increase the farm profitability as a consequence of reduced feeding costs.

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