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An *in vitro* evaluation of the potential use of greenhouse wastes to replace barley in goats' diets

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Abstract. The aim of this work was to evaluate the effect of replacing barley grain with greenhouse waste fruits (tomato and cucumber) in diets for ruminants on their *in vitro* rumen fermentation. A control diet with cereal straw, wheat bran, sunflower meal, alfalfa hay and barley grain was compared with diets in which barley grain was gradually replaced with tomato, cucumber or a mixture (1:1) of both. These diets were incubated in batch cultures using rumen fluid from goats as inoculum. Pressure and gas volume were measured at 0, 2, 4, 6, 12, 24, 48 and 72 h post-inoculation. Samples were collected after 24 h of incubation for volatile fatty acids (VFA) analyses. The type of waste did not affect kinetic (P = 0.658 and P = 0.885 for A and c, respectively) of gas production (GP) but increasing level of waste in the diet seemed to promote faster GP rate. Waste type and level of inclusion in the diet did not change pH (P ≥ 0.304), total VFA and most of VFA molar proportions. However, higher proportions of propionic acid (P = 0.023) were observed at the highest level of waste. Results from this experiment indicate that tomato, cucumber or a combination of both wastes can be included in ruminant diets to replace barley grain without compromising *in vitro* rumen fermentation.

Keywords. Barley – Batch cultures – Gas production – Greenhouse wastes – Ruminal fermentation.

Une évaluation *in vitro* de l'utilisation potentielle des déchets de serre pour remplacer l'orge dans régimes de chèvres

Résumé. *L'effet du remplacement de l'orge par des résidus des cultures sous serre (tomate et concombre) sur la fermentation ruminale a été évalué. Un régime composé de paille de céréales (30%), blé (5%), farine de tournesol (15%), foin de luzerne (22%) et d'orge (25%) a été comparé avec d'autres ou l'orge était (0, 25, 50, 75 et 100%) remplacés par la tomate, le concombre, ou une combinaison des deux. Les régimes ont été incubés dans des cultures fermés en utilisant de liquide du rumen des chèvres comme inoculum. La pression et le volume du gaz ont été mesurés après 0, 2, 4, 6, 12, 24, 48 et 72 h. A partir de 24 h, des échantillons ont été prélevés et analysés pour les acides gras volatils (AGV). Le type de résidu n'a pas affecté (P = 0.658 et P = 0.885 pour A et c, respectivement) la production du gaz (GP). Le type de résidu et le niveau n'ont pas changés (P ≥ 0.304) le pH, les VFA et les proportions molaires de la plupart des acides gras individuels. Les proportions les plus élevés (P = 0.023) de l'acide propionique ont été observés quand l'orge était remplacée au plus haut niveau. La tomate, le concombre ou une combinaison des deux peuvent être inclus dans l'alimentation des chèvres en remplaçant l'orge sans compromettre la fermentation ruminale.*

Mots-clés. *Orge – Cultures fermés – Production du gaz – Résidus des cultures sous serre – Fermentation ruminale.*

I – Introduction

In Mediterranean areas, there is a shortage of pastures that causes dependence of concentrate for animal feeding. Concentrates for ruminants are based mainly on cereals, whose prices have increased as a result of different factors (European Parliament, Plenary Session, January, 2009). Strategies to reduce concentrates in ruminant feeding are thus needed. Unconventional feedstuffs such as greenhouse wastes could represent an alternative providing environmental advantages. Spain is the European country with the highest surface devoted to greenhouses culture (Eumedia, 2008), producing important amounts of wastes (mainly tomato but also cucumber). The use of non conventional feedstuffs for ruminants requires the study of their effects on rumen fermentation and *in vitro* techniques may be used to avoid risks on animal health and allow evaluating a high number of samples (Kajikawa *et al*., 2003). To our knowledge, there is no information on the use of tomato or cucumber fruits in ruminants feeding. Only tomato pulp, a by-product from tomato processing industry, has been previously studied (Ojeda and Torrealba, 2001; Denek and Can, 2006). Therefore, the objective of the present work was to study the effect of replacing barley grain with greenhouse wastes (tomato and cucumber or a combination of both) in ruminants' diets, on their ruminal fermentation.

II – Material and methods

A control diet (CO) composed of cereal straw (30%), wheat bran (5%), sunflower meal (15%), alfalfa hay (22%) and barley grain (25%) was compared with others in which barley grain was gradually (20, 40, 60, 80 and 100 %) replaced with tomato (diets T1, T2, T3, T4 and T5), cucumber (diets C1, C2, C3, C4 and C5) or a mixture (1:1) of both (diets TC1, TC2, TC3, TC4 and TC5). All the diets contained 1% of a vitamin-mineral supplement. Tomato and cucumber from the Vegetable Wastes Processing Plant in Motril (Granada, Spain), were homogenized with a blender and kept at -20ºC until freeze-drying.

Three adult dry non-pregnant rumen-fistulated Granadina goats (46.9 ± 2.15 kg body weight (BW)) were used as inoculum donors. Animals were placed in individual boxes with free access to water and were fed a diet based on alfalfa hay supplied to meet maintenance energy requirements (Prieto *et al*., 1990). Rumen content from each goat was obtained immediately before the morning feeding, mixed and strained through four layers of cheesecloth.

Three identical incubation runs were carried out in batch cultures in three consecutive runs. The rumen fluid was mixed with a buffer solution (Goering and van Soest, 1970) in the proportion 1:4 (vol/vol) at 39ºC under continuous CO_2 flushing. In each run, duplicate samples of 0.5 g DM of each experimental diet were incubated into culture bottles of 120 ml with 50 ml of buffered ruminal fluid added under CO_2 flushing. In each run a total of 36 bottles (2 bottles for control and experimental diets, 2 blanks and 2 containing alfalfa hay as standard) were incubated. Bottles were sealed and incubated at 39ºC. In each bottle pressure and gas volume were measured at 2, 4, 6, 8, 12, 24, 48 and 72 hours after inoculation using a pressure transducer and a calibrated syringe, respectively. After 24 h of incubation, half of the bottles were uncapped, the pH was measured and one sample of bottle's content was collected for volatile fatty acid (VFA) analysis.

The dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP) content of diets ingredients were analysed according to the AOAC (2005). The neutral (NDF) and acid (ADF) detergent fibre were analysed according to van Soest *et al*. (1991) using an ANKOM^{220} Fiber Analyzer (Ankom Technology, Macedon, NY, USA). The α-amylase was used for NDF analysis. The *in vitro* dry matter and organic matter digestibility of the different diets' ingredients were determined according to the procedure described by Tilley and Terry (1963) using a DAISYII Incubator (Ankom Technology, Macedon, NY, USA). Total and individual VFA were analysed using a gas chromatography technique (Isac *et al*., 1994). The amount of VFA produced was obtained by subtracting the amount present in the rumen fluid at the start of inoculation from that obtained after 24 hours of incubation. The gas production pattern was adjusted to the model of France *et al*. (2000): $y = A\,[1 - e^{-c \cdot t}]$ where "y" represents the cumulative gas production (ml), "t" the incubation time (h), "A" the asymptote (total gas produced; ml) and "c" the gas production rate. Data were analysed as an unvaried model using the GLM procedure of SPSS software (SPSS® v.15, Chicago, IL, USA). Effects were considered significant at $P < 0.05$. When significant differences were detected, differences among means were tested using the Tukey's comparison test.

III – Results and discussion

Tomato and cucumber wastes had higher protein content (153 and 163 g/kg DM, respectively) than barley grain (90.4 g/kg DM) but NDF content in wastes and barley were similar (Table 1). Tomato fruits had the highest EE content maybe due to the presence of pigments (Abdel-Rahman, 1982).

Table 1. Chemical composition (g/kg dry matter) and *in vitro* digestibility (g/g) of diet ingredients

	Barley grain	Wheat bran	Tomato	Cucumber	Sunflower meal	Cereal straw	Alfalfa hay
Dry matter (g/kg fresh matter)	897	856	61.5	37.0	911	902	899
Organic matter	974	943	899	887	930	935	900
Neutral detergent fibre	185	395	191	168	445	621	400
Acid detergent fibre	112	191	139	131	223	357	267
Acid detergent lignin	56.2	90.1	48.7	2.50	37.0	4.00	14.0
Ether Extract	22.1	34.4	38.8	13.0	7.00	10.9	14.8
Crude protein	90.4	154	153	163	329	71.2	165
Tannins							
Free			4.71	15.0			
Fibre-bound			4.47	20.6			
Protein-bound			1.73	15.0			
Total†			10.9	50.6			
In vitro digestibility							
Dry matter	0.81	0.68	0.91	0.97	0.62	0.40	0.62
Organic matter	0.82	0.67	0.91	0.98	0.61	0.38	0.60

† Calculated as free + fibre-bound + protein-bound tannins.

Although tannins in cucumber were higher (1.5 g of protein-bound condensed tannins/100 g DM) than in tomato, ammonia or gas production did not seem to be different for diets based on those wastes. Since protein content is higher in diets based on cucumber as well, the reduction in protein degradability caused by tannins could be avoided by the extra amount of crude protein The average pH was within physiological values (Table 2). Both type ($P = 0.304$) or level of waste ($P = 0.947$) in diet did not affect pH.

The kinetics of gas production for experimental diets were similar (Fig. 1). The gas produced after 24 h of incubation (Table 2) ranged from 172, for control diet, to 188 ml/g incubated DM for diets including tomato and cucumber, which were close to the values found by Blummel *et al*. (1999) and Gierus *et al*. (2008) for grass silage, oat-whole crop silage and maize stover leaves (200, 214 and 201 ml/g DM, respectively). The rate of gas production was higher (from 0.078 to 0.099 ml/h) than values reported for shrubs such *Arbutus unedo* and *Quercus suber* (0.065 and 0.046 ml/h, respectively) by Gasmi-Boubaker *et al*. (2005), and also higher than those obtained for different oak species treated with PEG such as *Quercus persica*, *Quercus infectoria* and *Quercus libani* (0.054, 0.06 and 0.057 ml/h, respectively), studied by Elahi *et al*. (2008). There was not effect of the type of waste or the level of barley substitution either on gas production ($P \geq 0.689$) after 24 h of incubation or on its production rate ($P \geq 0.421$). However diets with the highest level of waste fruits showed an apparent faster fermentation rate than control diet (0.109 and 0.078, respectively). This is in concordance with Fondevila *et al*. (1994) who found a 15% increase in OM digestibility for a barley-based diet after supplementing with 300 g of tomato pomace/kg. Differences between our data and those from other authors from in vitro experiments could be related to the amount of substrate incubated and also to the inoculum characteristics (Molina-Alcaide *et al*., 1997). An increased rate of degradation, derived of wastes inclusion may indicate an increase intake of those diets in comparison with the control one.

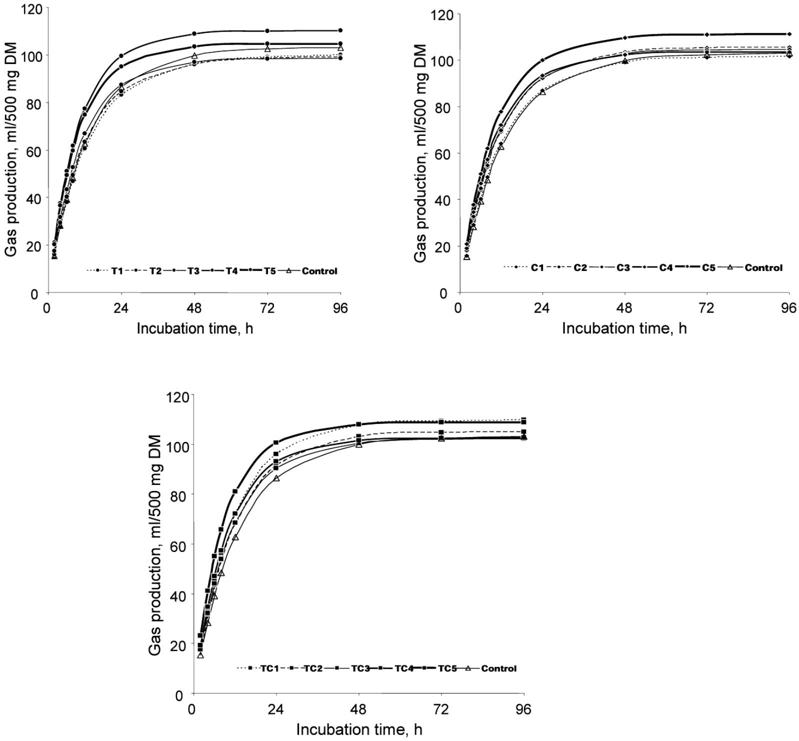

Fig. 1. Gas production curves at different times for control and experimental diets including increasing levels (20, 40, 60, 80 and 100) of tomato (T), cucumber (C) and tomato plus cucumber (TC) wastes.

The substitution of barley with tomato or cucumber did not affect the total VFA production (P = 0.243) which is in agreement with Cone and van Gelder (1999), who observed a good relationship between VFA and gas production. Only butyric acid production (P = 0.043) and the acetic/propionic ratio (P = 0.039) were affected by the type of waste included in diet. Since diets including cucumber had lower acetic/propionic acid ratio than the others it could also improve nitrogen retention as it was mentioned by Eskeland *et al*. (1974). The level of waste inclusion in diet affected propionic (P = 0.023) and valeric (P = 0.025) acids production. A tendency (P = 0.088) towards lower acetic/propionic ratio was observed as the level of barley substitution with wastes increased.

Table 2. Effects of type and level of greenhouse wastes inclusion in diet on average values of pH, gas and volatile fatty acids (VFA) production after 24 h of incubation in batch cultures

	Diet				**Waste level†**					**P-value††**			**SEM†††**
	CO	**T**	**C**	**T+C**	**20**	**40**	**60**	**80**	**100**	**D**	**WL**	**D x WL**	
pH	6.66	6.71	6.73	6.72	6.72	6.72	6.73	6.72	6.71	0.304	0.947	0.972	*0.057*
Ammonia (mg/l)	221	228	200	195	210	205	198	216	208	0.242	0.972	0.995	*7.29*
A (ml)	103	103	105	106	104	103	102	106	108	0.658	0.716	0.880	*1.33*
c (h^{-1})	0.078	0.094	0.094	0.099	0.084	0.089	0.095	0.103	0.109	0.885	0.421	0.989	*0.039*
Gas production (ml/g incubated DM)	172	179	185	188	177	178	179	190	196	0.766	0.689	0.994	*4.32*
VFA (µmol)													
Total VFA	2,412	3,248	3,084	3,194	3,129	3,158	3,067	3,324	3,198	0.243	0.343	0.948	*45.3*
Acetic	1,562	2,167	2,054	2,142	2,076	2,110	2,071	2,216	2,134	0.236	0.484	0.859	*32.4*
Propionic	404^{A}	558	564	552	544^{B}	539^{B}	523^{B}	595^{C}	588^{C}	0.827	0.023	0.785	*9.60*
Iso-Butyric	28.9	33.1	30.0	29.9	33.2	31.9	29.2	31.7	29.0	0.367	0.627	0.999	*0.88*
Butyric	329^{a}	391^{b}	352^{a}	380^{ab}	378	383	360	387	365	0.043	0.600	0.994	*6.20*
Iso-Valeric	45.2	53.3	45.8	48.0	50.9	50.0	46.6	50.9	46.7	0.166	0.837	0.996	*1.43*
Valeric	42.7^{AB}	44.5	37.9	41.2	46.8^{B}	44.2^{AB}	36.9^{A}	43.3^{AB}	34.9^{A}	0.120	0.025	0.297	*1.38*
Acetic / propionic	3.86^{ab}	3.89^{b}	3.67^{a}	3.89^{b}	3.83	3.92	3.97	3.73	3.65	0.039	0.088	0.380	*0.041*

† (20,40,60,80,100): percentage of greenhouse wastes in experimental diets.

†† D: diet; WL: waste level; D x WL: diet by waste level interaction.

††† SEM: standard error of the mean (n=48).

a,b Within a row diet means without a common superscript letter differ ($P < 0.05$).

A, B, C Within a row waste level means without a common superscript letter differ ($P < 0.05$).

IV – Conclusions

Substitution of barley grain with tomato, cucumber or a mixture of both wastes from greenhouse cultures in diets did not compromise the ruminal fermentation, indicating the potential of those wastes to replace cereals. The use of the highest level of tomato, cucumber or a mixture of both may be considered the most adequate as it tends to lower the acetate/propionate ratio and it could additionally involves an important reduction in ruminants feeding cost.

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