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Romero-Huelva M., Martín-García A.I., Nogales R., Molina Alcaide E.

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Effect of ash from different residual biomass used to obtain renewable energy on ruminal fermentation of diets based on wheat straw in batch cultures

M. Romero-Huelva, A. I. Martín-García, R. Nogales and E. Molina-Alcaide

Estación Experimental del Zaidín (CSIC), Profesor Albareda 1, 18008 Granada (Spain)

Abstract. The biomass-based renewable energy production generates big amounts of ashes. The recycling of these ashes is of environmental and economical importance. The aim of the present work was to study the effect of adding ash from different residual biomass (wood, olive oil extraction, winery and greenhouses wastes) on ruminal fermentation of wheat straw (WS) and wheat straw plus concentrate (WSC). Batch cultures of mixed rumen micro-organisms were used. The ruminal inoculum was obtained from 3 rumen-fistulated Granadina goats. The kinetic of gas production (GP) was evaluated by following an exponential model $y = A [1 - e^{-c \cdot t}]$. Significant effect ($P < 0.05$) was found on the ratio of acetate to propionate and on total volatile fatty acid (VFA) and acetate production after 24 h of WS incubation, as well as on ammonia concentration after 72 h of WSC incubation. The average values for those parameters were higher for both diets with ashes from greenhouses. The inclusion of ashes from biomass-based renewable energy production did not or only slightly affect the *in vitro* ruminal fermentation of wheat straw without or with concentrate.

Keywords. Ash – Residual biomass – Ruminal fermentation – Methane.

Effet des cendres de diffèrent biomasses résiduels utilisées pour obtenir de l'énergie renouvelable sur la fermentation ruminale des régimes á base de la paille de céréales dans des cultures fermés

Résumé. *La procédure pour la production d'énergie renouvelable à partir de biomasse résiduelle produit aussi de grandes quantités de cendres donc le recyclage est important pour l'environnement et l'économie. L'objectif du présent travail était d'étudier l'effet de l'ajout de cendres provenant de différents types de biomasse résiduelle (bois, sous-produits de l'extraction de l'huile d'olive, résidus des cultures sous serre) sur la fermentation ruminale de la paille de blé (WS) et de la paille de blé plus concentré (WSC). Des cultures fermés de micro-organismes du rumen ont été utilisées. Le inoculum a été obtenu à partir du contenu du rumen des chèvres de race Granadina avec des canules du rumen. La cinétique de la production de gaz (GP) a été évaluée selon le modèle exponentiel* $y = A [1 - e^{-c \cdot t}]$. *Des effets significatifs* ($P < 0,05$) *ont été observés pour les valeurs acétique/propionique, la production des acides gras volatiles (VFA) et d'acétique après 24 h d'incubation de WS aussi que sur la concentration de l'ammoniaque après 72 d'incubation de WSC. Les valeurs moyennes de ces paramètres étaient plus hautes pour les deux régimes seulement quand des cendres de sous-produits des cultures sous serre étaient ajoutées. Les autres cendres n'affectaient pas la fermentation ruminale.*

Mots-clés. *Cendres – Biomasse résiduelle – Fermentation ruminale – Méthane.*

I – Introduction

Fifty per cent of renewable energy is being produced from residual biomass. Both the "Energy Plan" approved by the European Council in 2008 and the "Renewable Energy Plan" approved by the Spanish Government for the period 2005-2010 focus on increasing the use of this type of energy. However, the procedure generates important amounts of ash (2.5-5% of processed residual biomass, dry matter basis) as stated by García Almiñana and Solé Xam-mar (2005). Since the storage of ash causes environmental and economical problems the recycling is of great interest. Minerals are essential nutrients for animals as elements for structural tissues and metabolic reaction as well as elements in ruminal microbial activity (Ammerman and Goodrich, 1983; Anke *et al*., 1998). Microminerals also play an important role in rumen protozoa and bacterial growth and metabolism. The aim of this work is to evaluate the effect of addition ash from renewable energy production based on different residual biomass on the ruminal fermentation of diets based on wheat straw in batch cultures of mixed rumen micro-organisms.

II – Materials and methods

Batch cultures of mixed rumen micro-organisms (Theodorou *et al*., 1994) were used to study the gas production promoted by the fermentation of two experimental diets composed of wheat straw (diet WS) and a 70:30 (OM basis) mixture of WS and a concentrate (diet WSC) composed of barley grain and faba beans in a 5:3 ratio. Two identical incubation runs were carried out in two consecutive weeks. In each run 120 ml glass bottles were used with triplicate samples of 500 mg OM of the experimental diet (WS or WSC) without or with (10 mg) ash from different residual biomass (wood, olive oil extraction, winery and greenhouses wastes coming from different pats of Spain) were incubated for 24 and 72 h, respectively. The composition of ashes is shown in Table 1.

Table 1. Mineral composition of ashes (n = 3)

g/kg	C	N	P	K	Ca	Mg	Na	Fe
W1	82.1	0.360	1.69	27.7	147	20.6	5.48	6.29
W2	18.4	0.195	1.02	6.4	63.7	9.73	2.42	16.6
O1	–	–	17.5	20.1	150	45.2	3.23	17.9
O2	24.2	1.13	4.17	57.8	52.2	15.1	2.83	6.28
WN	–	1.78	14.3	31	184	10.1	6.28	3.25
GH	63.9	2.01	2.66	30.7	60.8	18.1	8.23	9.76
mg/kg		**Mn**	**Cu**	**Zn**	**Cd**	**Ni**	**Pb**	**B**
W1		4635	48	110	<0.2	52	6	220
W2		386	247	2064	<0.2	52	432	205
O1		438	207	183	–	–	–	–
O2		172	181	274	<0.2	30	67	560
WN		167	85	17	–	–	–	–
GH		369	88	169	<0.2	14	2	176

W1: residual wood ash from Huelva; W2: residual wood ash from Jaén; O1: olive wastes ash from El Tejar (Córdoba); O2: olive wastes ash from Jaén; WN: winery wastes ash from Ciudad Real; GH: greenhouse wastes ash from Almería.

Three adult dry non-pregnant rumen-fistulated Granadina goats (46.9 ± 2.15 kg body weight (BW)) were fed alfalfa hay supplied to meet energy maintenance 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 as described by Theodorou *et al*. (1994). The first day of each incubation run, rumen liquor and buffer solution (Goering and van Soest, 1970) were mixed in a 1:4 ratio and used as inoculum (50 ml/bottle). In each run a blank was also incubated. Bottles were sealed and incubated at 39ºC. Pressure in the bottle headspace and volume of gas produced were measured, at 2, 4, 6, 8, 12, 24, 48 and 72 h after inoculation. After 24 and 72 h of incubation an aliquot of the gas produced was sampled for CH_4 analysis, bottles were open and the pH was measured and then fermentation was stopped introducing the bottles in ice. The weight of the bottle content after fermentation period was recorded and two sub-samples were collected. One ml of that content was added to one ml of deproteinising solution composed of 20 g of metaphosphoric acid and 4 g of crotonic acid, as internal standard, per litre of 0.5 M-HCl, for volatile fatty acid (VFA) analysis. For ammonia-N analysis 1 ml was also taken and 1 ml of 0.5 M-HCl was added.

The gas production, corrected for that in the blanks 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 or maximal gas production (ml) and, "c" the gas production constant rate (h^{-1}). The amount of VFA produced after the fermentation period was corrected by subtracting the amount in the inoculum prior to incubation.

Ground (1-mm) samples of ingredients were analyzed for dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP) according to the AOAC (2005). The NDF and ADF were analyzed according to van Soest *et al*. (1991) using an ANKOM Model 220 Fiber Analyzer (Macedon, NY, USA) with amylase for NDF analysis and NDF and ADF contents referred to ash-free weight. The ADL was determined by solubilisation of cellulose with 72% sulphuric acid. The acid detergent insoluble N (ADIN) was determined by combustion of ADF residues using a Leco TruSpec CN® (St. Joseph, MI, USA). The energy content was determined using an oxygen bomb calorimeter (PARR 1356, Biometer®). Mineral composition of ashes was analyzed by atomic absorption spectrophotometry (Perkin Elmer HGA500, USA), after percloric and nitric acid digestion of the sample. The carbon and nitrogen content of ashes was determined by Leco TruSpec CN® (St. Joseph, MI, USA).

The concentration of NH_3-N was determined colorimetrically using the phenol-hypochlorite method (Weatherburn, 1967). Total and individual VFA were analyzed by gas chromatography (Isac *et al*., 1994).

Data were analyzed by one-way ANOVA procedure of SPSS 15.0® (Chicago, IL, USA). Differences between runs were considered as random effect. The effects were considered significant at $P < 0.05$. Differences among means were tested using the Tukey comparison test.

III – Results and discussion

The chemical composition of diet ingredients is shown in Table 2.

Table 2. Chemical composition (g/100g dry matter) and gross energy (GE) of diets ingredients (n = 3)

	Wheat straw	Barley	Faba bean
Organic matter	91.6	97.6	96.4
Ether extract	0.662	1.78	1.03
NDF	73.4	20.5	23.0
ADF	43.9	5.32	11.6
ADL	6.07	1.52	1.21
Crude protein	3.45	8.79	23.2
ADIN (g/100 g total N)	38.4	53.2	16.1
Gross energy (MJ/kg dry matter)	16.6	17.0	17.4

The average pH values, gas production, and ammonia and methane (CH_4) concentration after 24 h of WS incubation were not affected ($P > 0.05$) by the addition of ash (Table 3). The total VFA production was lower ($P < 0.05$) for WS added with O2 than for WS added with GH ash. The molar proportion of acetate was higher ($P < 0.05$) for WS added with GH ash than for WS added with W2, O1, O2 or WN ash. The acetate/propionate ratio was higher ($P < 0.05$) for WS added with GH ash in comparison with NA and WS added with the other ashes. These higher values might be due to differences in the mineral composition of ashes that might have influenced the microbial growth and metabolism. In fact the production of volatile fatty acids by rumen microbes is generally influenced by macro minerals as reported by Durand and Komisarczuk (1988) and Komisarczuk-Bony and Durand (1991). Giraldo *et al*. (2007) found similar results concerning gas and VFA production and ammonia concentration after 24 h of wheat straw incubation.

Table 3. Effect of the addition of different ashes† on the fermentation parameters after 24 h of wheat straw incubation in batch cultures

	NA	W1	W2	O1	O2	WN	GH	*SEM*††	P-value
pH	6.75	6.74	6.72	6.73	6.73	6.74	6.75	0.009	0.192
NH_4 (mg/100 ml)	16.8	16.7	18.4	16.1	18.3	16.6	18.7	1.289	0.618
Gas prod (ml/g incubated OM)	110	110	110	110	107	108	111	2.569	0.901
CH_4 (ml/100 ml gas)†††	10.3	10.3	10.3	10.3	10.2	10.3	10.5	0.124	0.881
Total VFA (mmol/d)	1.56^{ab}	1.54^{ab}	1.51^{ab}	1.52^{ab}	1.48^{a}	1.51^{ab}	1.76^{b}	0.068	0.019
VFA (mol/100 mol)									
Acetate	64.2^{abc}	64.1^{abc}	63.4^{a}	63.8^{a}	63.8^{a}	63.6^{a}	67.3^{c}	0.058	0.012
Propionate	25.9	25.9	26.4	26.1	26.1	26.2	23.6	0.010	0.473
Iso-Butyrate	0.288	0.287	0.298	0.290	0.283	0.319	0.304	0.001	0.198
Butyrate	8.01	8.07	8.27	8.16	8.18	8.29	7.33	0.003	0.672
Iso-Valerate	0.129	0.141	0.151	0.154	0.150	0.167	0.171	0.001	0.522
Valerate	1.42	1.45	1.49	1.47	1.49	1.45	1.29	0.001	0.350
Acetate/propionate	2.48^{a}	2.47^{a}	2.40^{a}	2.45^{a}	2.45^{a}	2.43^{a}	2.84^{b}	0.102	0.006

† NA: diet without ash; W1: residual wood ash from Huelva; W2: residual wood ash from Jaén; O1: olive wastes ash from El Tejar (Córdoba); O2: olive wastes ash from Jaén; WN: winery wastes ash from Ciudad Real; GH: greenhouse wastes ash from Almería.

†† *SEM*: standard error of the mean (n=6).

††† Methane concentration of gas produced between 12 and 24 h of incubation.

No effect ($P > 0.05$) of ashes was found on any of the fermentation parameters after 72 h of WS incubation (Table 4). Gas production and methane concentration were higher than values found by Varadyová *et al*. (2006), but VFA production was lower than those observed by these authors. Differences observed between our data and those from Varadyová could be related to the amount of substrate incubated and to the characteristics of ruminal liquor used as inoculum (Molina *et al*., 1997).

No effect ($P > 0.05$) of ash addition was found on the average values of WSC fermentation parameters after 24 h of incubation (Table 5). However ammonia concentration after 72 h of WSC incubation increased ($P < 0.05$) with the addition of GH ash (Table 6). A trend to an increase in gas production ($P = 0.063$) and in the rate of gas production ($P = 0.089$) with addition of GH ash was found. Ammonia concentration after 24 and 72 h of WS and WSC incubation was within the range of values considered as optimal for ruminal microbial growth (Mehrez *et al*., 1997).

The addition of ashes from different residual biomass (wood, olive, greenhouse and winery) to WS and WSC did not modify ($P \geq 0.089$) the gas production.

Table 4. Effect of the addition of different ashes† on the fermentation parameters after 72 h of wheat straw incubation in batch cultures

	NA	W1	W2	O1	O2	WN	GH	*SEM*††	P-value
pH	6.64	6.65	6.64	6.65	6.65	6.67	6.67	0.009	0.064
NH_4 (mg/100 ml)	27.5	28.7	29.4	31.9	31.6	28.9	25.6	1.942	0.195
Gas prod (ml/g incubated OM)	228	228	228	227	226	226	229	1.787	0.847
CH_4 (ml/100 ml gas)†††	14.3	14.7	14.3	14.2	14.2	14.4	14.3	0.248	0.724
A (ml)	157	158	157	156	162	161	158	3.365	0.834
c (h^{-1})	0.018	0.018	0.018	0.018	0.017	0.017	0.018	0.001	0.921
Total VFA (mmol/d)	0.928	0.988	0.972	0.926	0.935	0.939	0.892	0.029	0.213
VFA (mol/100 mol)									
Acetate	62.8	62.9	62.4	62.6	62.0	61.8	61.7	0.022	0.313
Propionate	22.9	22.1	23.3	24.1	22.4	23.0	23.5	0.008	0.634
Iso-Butyrate	1.88	1.72	1.84	1.56	2.32	1.77	1.47	0.003	0.376
Butyrate	8.61	8.54	8.57	8.38	8.35	8.52	8.52	0.004	0.537
Iso-Valeriate	1.93	2.38	2.03	1.75	2.32	2.33	2.33	0.003	0.506
Valeriate	1.87	2.37	1.83	1.62	2.58	2.64	2.42	0.004	0.325
Acetate/propionate	2.74	2.85	2.70	2.61	2.77	2.70	2.62	0.097	0.518

† NA: diet without ash; W1: residual wood ash from Huelva; W2: residual wood ash from Jaén; O1: olive wastes ash from El Tejar (Córdoba); O2: olive wastes ash from Jaén; WN: winery wastes ash from Ciudad Real; GH: greenhouse wastes ash from Almería.
†† *SEM*: standard error of the mean (n=6).
††† Methane concentration of gas produced between 12 and 24 h of incubation.

Table 5. Effect of the addition of different ashes† on the fermentation parameters after 24 h of wheat straw plus concentrate incubation in batch cultures

	NA	W1	W2	O1	O2	WN	GH	*SEM*††	P-value
pH	6.71	6.72	6.74	6.72	6.73	6.73	6.73	0.009	0.250
NH_4 (mg/100 ml)	18.5	16.9	18.0	20.1	18.5	22.0	20.1	2.012	0.555
Gas prod (ml/g incubated OM)	154	150	152	154	153	154	170	5.469	0.060
CH_4 (ml/100 ml gas)†††	12.2	12.2	12.2	12.3	12.2	12.3	12.2	0.084	0.757
Total VFA (mmol/d)	1.92	1.92	1.89	1.92	1.98	1.95	1.87	0.074	0.943
VFA (mol/100 mol)									
Acetic	61.3	60.6	60.4	61.1	60.7	60.9	60.6	0.059	0.977
Propionate	22.0	22.2	22.3	22.2	22.5	22.2	22.3	0.011	0.546
Iso-Butyrate	0.534	0.570	0.556	0.537	0.545	0.550	0.542	0.001	0.419
Butyrate	14.0	14.3	14.6	14.0	14.2	14.1	14.3	0.007	0.808
Iso-Valerate	0.608	0.684	0.647	0.624	0.640	0.638	0.627	0.001	0.238
Valerate	1.54	1.58	1.59	1.54	1.54	1.55	1.58	0.001	0.125
Acetate/propionate	2.78	2.72	2.70	2.75	2.69	2.73	2.71	0.085	0.993

† NA: diet without ash; W1: residual wood ash from Huelva; W2: residual wood ash from Jaén; O1: olive wastes ash from El Tejar (Córdoba); O2: olive wastes ash from Jaén; WN: winery wastes ash from Ciudad Real; GH: greenhouse wastes ash from Almería.
†† *SEM*: standard error of the mean (n=6).
††† Methane concentration of gas produced between 12 and 24 h of incubation.

Table 6. Effect of the addition of different ashes† on the fermentation parameters after 72 h of wheat straw plus concentrate incubation in batch cultures

	NA	W1	W2	O1	O2	WN	GH	*SEM*††	P-value
pH	6.67	6.67	6.68	6.68	6.68	6.68	6.67	0.013	0.974
NH_4 (mg/100 ml)	31.6[a]	31.5[a]	32.3[a]	33.2[a]	36.0[ab]	40.8[ab]	46.3[b]	2.795	0.001
Gas prod (ml/g incubated OM)	272	270	268	269	268	272	283	4.022	0.063
CH_4 (ml/100 ml gas)†††	15.5	15.6	15.4	15.5	15.6	15.6	16.2	0.232	0.112
A (ml)	157	159	155	155	154	158	156	1.485	0.130
c (h^{-1})	0.028	0.027	0.028	0.029	0.029	0.028	0.033	0.002	0.089
Total VFA (mmol/d)	0.988	1.03	0.981	0.983	1.03	1.03	1.05	0.045	0.844
VFA (mol/100 mol)									
Acetate	57.6	58.2	58.3	57.4	58.6	58.4	59.3	0.032	0.784
Propionate	22.1	22.0	22.0	22.2	21.7	21.8	20.0	0.012	0.943
Iso-Butyrate	1.83	1.17	1.55	1.39	1.88	1.78	2.01	0.002	0.064
Butyrate	13.9	13.9	13.3	13.9	13.0	13.2	13.6	0.007	0.817
Iso-Valerate	2.39	2.64	2.58	2.67	2.59	2.53	2.80	0.003	0.928
Valerate	2.16	2.16	2.29	2.40	2.15	2.20	2.35	0.005	0.999
Acetate/propionate	2.62	2.66	2.68	2.59	2.72	2.70	2.97	0.136	0.445

† NA: diet without ash; W1: residual wood ash from Huelva; W2: residual wood ash from Jaén; O1: olive wastes ash from El Tejar (Córdoba); O2: olive wastes ash from Jaén; WN: winery wastes ash from Ciudad Real; GH: greenhouse wastes ash from Almería.

†† *SEM*: standard error of the mean (n=6).

††† Methane concentration of gas produced between 12 and 24 h of incubation.

The values obtained for *A* were higher than those found by Khelil (2008) with good quality diets. Differences might be due to the different procedure used in both works to obtain the inoculum as the procedure followed in the present work (Theodorou *et al*., 1994) may ensure the presence of more diverse and numerous microbial communities, both fibre-associated and free microorganisms, than the one followed by Khelil (2008). However, our results showed slower gas rate production than values obtained by Khelil (2008) may be due to higher fiber content in the experimental diets of the present experiment, in comparison with those incubated by Khelil (2008).

IV – Conclusions

The results of this experiment indicate that inclusion of ashes from biomass-based renewable energy production did not or only slightly modify the *in vitro* ruminal fermentation of wheat straw without or with concentrate.

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