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Chemical composition and *in vitro* rumen fermentation of crude olive cake and olive extracts

C.N. Marcos[1], S. Chávez[1], C. Blas[1], E. Molina-Alcaide[2], M.J. Ranilla[3] and M.D. Carro[1]

[1]Departamento de Producción Agraria, E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid (Spain)
[2]Estación Experimental del Zaidin (CSIC), Profesor Albareda, 1, 18008 Granada (Spain)
[3]Departamento de Producción Animal, Universidad de León, 24071 León (Spain), Instituto de Ganadería de Montaña (CSIC), Finca Marzanas s/n, 24346 Grulleros, León (Spain)

Abstract. The aim of this study was to assess the nutritive value for ruminants of five samples of crude olive cake (COC) and two commercial olive extracts (Prolivols^{TM} and Hytaolive^{TM}). Alfalfa hay (AH) and barley straw (BS) were also evaluated for comparative purposes. Chemical composition was analysed in all samples. Gas production kinetic was determined in 144-h *in vitro* incubations with sheep rumen fluid, whereas fermentation parameters and *in vitro* dry matter digestibility (IVDMD) were analysed after 24 h of incubation. Crude protein, neutral detergent fibre (NDF), acid detergent fibre (ADF) and ether extract content of COC ranged from 65.2 to 105, 374 to 448, 269 to 316, and 145 to 267 g/kg dry matter (DM), respectively. The high amount of nitrogen bound to the ADF in the COC samples (25 to 45% of total N) indicated low N availability, and the lignin⁄NDF ratios were high (0.272 to 0.401). The IVDMD values of COC (47.9 to 60.8%) were lower than that for AH (67.5%), but greater than that for BS (42.4%). Potential gas production values of COC samples (60.3 to 103 ml/g DM) were lower (P<0.05) than those for olive fruits extracts, AH and BS (values > 170 ml/g DM). There were no differences (P>0.05) among COC samples in total volatile fatty acid (VFA) production after 24h of incubation, and values were similar to those of BS, but lower (P<0.05) than those measured for the two commercial extracts and AH. The COC could be used in ruminant diets replacing low-quality feeds such as BS, but due to its high ether extract content it could be also used in dairy animals to increase the content of unsaturated fatty acids in animal products.

Keywords. Olive cake – Olive extracts – Chemical composition - Gas production – Ruminal fermentation.

Composition chimique et fermentation ruminal* in vitro *des grignos d'olive et des extraits d'olive

Résumé. *L'objectif de ce travail a été d'estimer la valeur nutritive pour des animaux ruminants de cinq échantillons de grignon d'olive (COC) et deux extraits commerciaux (Prolivols^{TM} and Hytaolive^{TM}). Foin de luzerne (AH) et paille d'orge (BS) ont aussi été utilisés pour comparer. La cinétique de la production de gaz a été déterminée en incubant* in vitro *pendant 144 heures avec fluide du rumen de brebis. Les paramètres de la fermentation et la digestibilité de la matière sèche* in vitro *(IVDMD) ont été analysées 24 heures après que l'incubation a été commencée. Le contenu en matières azotées totales, fibre au détergent neutre (NDF), fibre au détergent acide (ADF) et gras des échantillons de COC varié entre 65,2 et 105, 374 et 448, 269 et 315 et 145 et 267 g/kg matière sèche (DM), respectivement. La grande quantité d'azote lié à l'ADF dans les échantillons de COC (25 à 45% des matières azotées totales) montre une faible disponibilité de l'azote ; le rapport lignine/NDF était haut (0,272 à 0,401). Les valeurs pour l'IVDMD des échantillons de COC (47,9 à 60,8%) étaient plus petites que la valeur pour l'AH (67,5%), mais plus grande que la valeur pour BS (42,4%). Les valeurs pour la production potentiel de gaz des échantillons de COC (60,3 à 103 ml/g DM) étaient plus petite (P<0,05) que les valeurs des extraits commerciaux, et celles pour AH et BS (valeurs > 170ml/g DM). Il n'y avait aucune différence (P > 0,05) entre les échantillons de COC pour la production des acides gras volatiles (VFA) totales, etant les valeurs similaires à la valeur pour BS, mais ils étaient plus petites (P<0,05) que les valeurs des extraits commerciaux et l'AH. Le COC pourrait être utilisé pour l'alimentation des animaux ruminants remplaçant des aliments fibreux, comme la BS, mais grâce à sa forte teneur en gras le COC pourrait être aussi utilisé pour l'alimentation des animaux laitiers á fin d'améliorer le contenu d'acides gras insaturés chez les produits d'origine animale.*

Mots-clés. *Grignon d'olive – Extraits d'olive – Production de gaz – Composition chimique – Fermentation ruminal.*

I – Introduction

Spain is the largest producer of olive oil in the world, and as a consequence produces a large volume of by-products causing serious environmental problems. A waste product with high moisture and pollution potential, called “alperujo”, is generated in the two-phases extraction procedure currently used by the oil industry (Molina and Yáñez-Rúiz, 2008). “Alperujo” is stored in ponds until further processing, which consists in removing olive stones and drying the residue off. The result of this process is a by-product called crude olive cake (COC) that could be used for ruminants feeding. Olives are rich in polyphenolic compounds and only a small part of them are extracted with the oil, most of polyphenols remaining in the waste products (Rodis *et al.,* 2000). Several extracts rich in polyphenols are commercially available and some studies have shown that adding olive extracts during meat processing delayed the oxidation process and increased the shelf-life of meat (Muíño *et al*., 2017). However, to our best knowledge there is no information on the ruminal degradation of these products when they are included in the feed. The objective of this study was to assess the chemical composition and *in vitro* ruminal fermentation of five different samples of COC and two commercial olive extracts.

II – Material and methods

Five samples of COC, obtained at different “almazaras”, and two commercial extracts (Prolivols™ and Hytaolive™) obtained from olives by physical extraction processes were used in this study. According to the manufacturer, Prolivols™ contains 35% of total polyphenols (particularly hydroxytyrosol and tyrosol), and Hytaolive™ has a high level of hydroxytyrosol. In addition, two forages widely used in ruminants feeding (alfalfa hay and barley straw) were included for comparative purposes. All substrates were grounded to 1 mm and their chemical composition was analysed according to the AOAC (1999). Neutral (NDF) and acid (ADF) detergent fibre and lignin were analysed according to Van Soest *et al*. (1991).

Substrates were fermented *in vitro* to determine gas production kinetics and ruminal fermentation parameters. Samples (200 mg of dry matter (DM)) of each substrate were weighed into 60-mL bottles. Ruminal fluid was obtained from four rumen-cannulated Lacaune sheep (64.7 ± 2.10 kg body weight) fed grass hay and concentrate in 2:1 proportion twice daily. Sheep were managed according to the protocols approved by the Institutional Animal Care and Use Committee of the Technical University of Madrid and had free access to water over the trial. Ruminal contents of each sheep were obtained immediately before the morning feeding and strained through four layers of cheesecloth. Fluid of each sheep was 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. Bottles were prewarmed (39ºC) prior to the addition of 20 ml of buffered rumen fluid, sealed with rubber stoppers and aluminium caps and incubated at 39ºC. Bottles without sample (blanks) were added to correct for endogenous gas production. Two incubations runs were performed. In the first run, gas production were measured at 3, 6, 9, 12, 18, 24, 36, 48, 72, 96 and 144 h using a pressure transducer (Widereager Wide Range Pressure Meter, Sper Scientific LTD, Scottsdale, AZ, USA) and a calibrated syringe, releasing the gas produced at each measurement time. In the second run, bottles were incubated for 24 h, gas production was measured as described before and a gas sample (10 ml) was stored in a vacuum tube for CH_4 analysis before taking samples for volatile fatty acid (VFA) and NH_3-N analyses as described by Martínez *et al*. (2010). Procedures for CH_4, VFA and NH_3-N analyses have been also described by Martínez *et al*. (2010). The amount of OM apparently fermented (OMAF) in each bottle was estimated from VFA production as described by Demeyer (1991).

In vitro DM digestibility (IVDMD) was determined by weighting 300 mg of substrate in polyester bags (30 µm pore size; Ankom Corp #57, Ankom Technology Corp., Fairport, NY, USA) which were incubated with buffered ruminal fluid in an Ankom Daisy II incubator (Ankom Technology Corp, Fairport,

NY, USA) at 39ºC under continuous rotation. After 24 h, bags were washed with cold water and dried at 60ºC for 48 h and weighted to calculate the IVDMD. Three bags were used for each substrate.

Gas production data were fitted with time using the exponential model: gas = $A\ (1 - e^{-(-c\ (t - lag))})$, where *A* is the asymptotic gas production (mL), *c* is the fractional rate of gas production (h^{-1}), *lag* is the initial delay in the onset of gas production (h) and t is the gas measurement time. The parameters *A*, *c* and *lag* were estimated by an iterative least squares procedure using the NLIN procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC, USA). The average gas production rate (AGPR; mL gas/h) was calculated as AGPR = $A\ c\ /\ [2\ (\ln 2 + c\ lag)]$. Data were analysed as a mixed model using the PROC MIXED of SAS. The effect of substrate was considered fixed and that of the inoculum as random. Significance was declared at $P < 0.05$, and comparison of means was performed by the Tukey test.

III – Results and discussion

Content of crude protein (CP), NDF, ADF, lignin and ether extract in COC samples ranged from 65.2 to 105, 274 to 448, 269 to 316, 122 to150, and 145 to 267 g/kg DM, respectively (Table 1). These values are in the range of those reported by others for COC of different sources (Molina-Alcaide *et al*., 2003; Molina-Alcaide and Yáñez-Ruiz, 2008).

Table 1. Chemical composition (g/kg dry matter) and *in vitro* dry matter digestibility (IVDMD) of crude olive cake (COC) samples, olive extracts (Prolivols and Hytaolive) and forages

Item[1]	COC1	COC2	COC3	COC4	COC5	Prolivols[2]	Hytaolive[2]	Alfalfa hay	Barley straw
CP	66.7	65.2	92.2	90.0	105	100	3.51	194	55.6
NDF	423	448	422	374	414	–	–	394	697
ADF	283	302	287	269	316	–	–	215	388
Lignin	123	122	144	150	150	–	–	45.0	38.8
CP-ADF	21.8	16.3	29.4	34.6	47.7	–	–	66.2	19.2
Ether extract	187	177	203	145	267	78.7	121	47.2	28.5
IVDMD (%)	59.3	60.8	60.6	54.5	47.9	–	–	67.5	42.4

[1] CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre.

[2] Due to the small particle size of these samples, some analyses could not be performed.

The high amount of CP bound to the ADF in the COC samples (25 to 45% of total CP) indicated low N availability, as previously reported (Molina-Alcaide and Yáñez-Ruiz, 2003). The IVDMD of COC samples (47.9 - 60.8%) was lower compared with alfalfa hay (67.5%) but greater than that of barley straw (42.4%). Due to the small particle size of the commercial extracts, fibre analyses could not be performed. Crude protein content in Prolivols was about 3 times greater than that in Hytaolive, but its ether extract was lower (78.7 *vs.* 121 g/kg DM).

Accumulated gas production was greater ($P<0.05$) for both olive extracts than for COC samples (Figure 1 and Table 2). The high gas production values observed for the two olive extracts would indicate an extensive fermentation in the rumen, as cumulative gas production is directly related with the amount of organic matter fermented (Menke *et al*., 1979). The high VFA production (Table 3) observed for Hytaolive also suports this hypothesis. The COC1 had greater ($P<0.05$) A values than the rest of COC, but there were no differences ($P>0.05$) among COC samples either in lag or AGPR values. There were no differences ($P>0.05$) among COC samples either in VFA and CH_4 production or NH_3-N concentrations, but COC4 showed greater ($P<0.05$) acetate:propionate ratios than the rest of COC samples (Table 3).

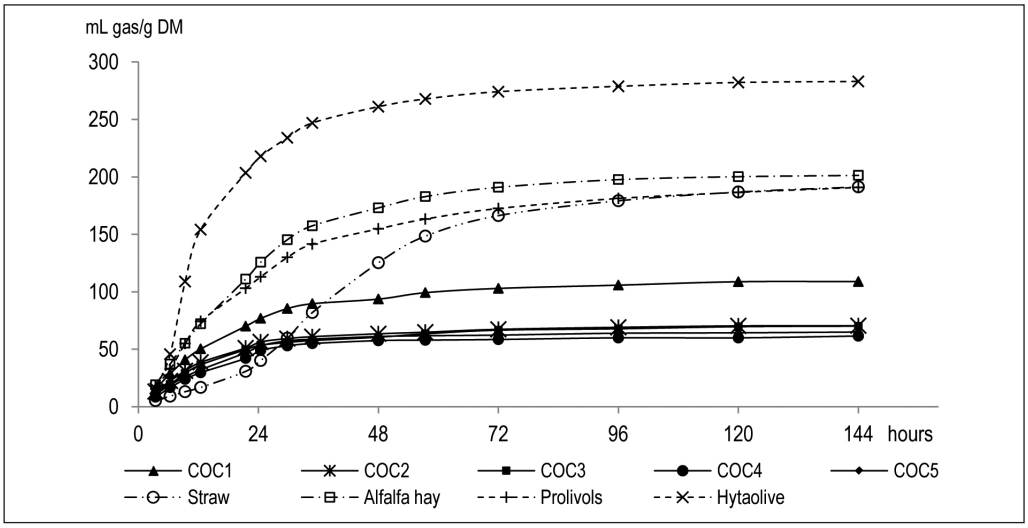

Fig. 1. ***In vitro*** **cumulative gas production of crude olive cake (COC) samples, olive extracts (Prolivols and Hytaolive) and forages.**

Table 2. Gas production parameters of crude olive cake (COC) samples, olive extracts (Prolivols and Hytaolive) and forages (n = 4)[1]

Substrate[1]	A	c	*lag*	AGPR
COC1	103^{b}	0.0533^{ab}	1.29^{a}	3.68^{a}
COC2	68.4^{a}	0.0695^{c}	0.68^{a}	3.24^{a}
COC3	67.7^{a}	0.0617^{bc}	0.47^{a}	2.90^{a}
COC4	60.3^{a}	0.0694^{bc}	1.61^{ab}	2.60^{a}
COC5	64.3^{a}	0.0678^{bc}	0.94^{a}	2.90^{a}
Prolivols	187^{c}	0.0402^{a}	0.96^{a}	5.19^{b}
Hytaolive	277^{d}	0.0740^{c}	3.04^{b}	11.2^{c}
sem[2]	4.21	0.00346	0.325	0.252
P =	<0.001	<0.001	<0.001	<0.001
Forages				
Alfalfa hay	202	0.0442	1.82	5.73
Barley straw	198	0.0303	15.4	2.57

[a-d] For COC and olive extracts, means in the same column with different superscript differ (P<0.05).

[1] A: potential gas production; *c*: fractional rate of gas production; *Lag*: time until the production of gas begins; AGPR: gas production rate until it has reached half of the A value.

[2] sem: standard error of the mean.

All COC samples had greater cumulative gas production values over the first 24 h of incubation compared with barley straw (Figure 1), which is consistent with the greater *lag* values of barley straw (15.4 h) compared with COC samples (0.47 to 1.61 h). However, CH_4 and VFA production values were numerically lower for COC (ranging from 173 to 199 µmol and from 604 to 688 µmol, respectively) than those for barley straw (250 and 733 µmol, respectively). Hytaolive produced more total VFA and CH_4 and had a greater amount of OMAF (P<0.05) than all COC samples and Prolivols (Table 3), which is consistent with the greater values of *A*, *c* and AGPR observed for Hytaolive compared with the rest of the samples tested.

Table 3. ***In vitro*** **fermentation parameters after 24 h incubation of crude olive cake (COC) samples, olive extracts and forages with buffered ruminal fluid from sheep (n = 4)[1]**

Substrate	CH_4 (µmol)	NH_3-N (mg/L)	Volatile fatty acid (VFA; µmol)					Ac/Pr (mol/mol)	OMAF (mg)
			Total	Ac	Pr	Bt	Minor VFA		
COC1	199[a]	304[b]	687[a]	382[a]	161[a]	103[a]	41.3[ab]	2.46[b]	60.6[a]
COC2	177[a]	337[b]	610[a]	328[a]	140[a]	95.5[a]	45.5[b]	2.41[b]	53.4[a]
COC3	173[a]	351[b]	604[a]	327[a]	137[a]	93.5[a]	47.1[b]	2.46[b]	52.7[a]
COC4	186[a]	364[b]	608[a]	344[a]	130[a]	88.8[a]	45.2[ab]	2.72[c]	52.8[a]
COC5	181[a]	339[b]	688[a]	370[a]	161[a]	105[a]	52.0[b]	2.34[b]	60.0[a]
Prolivols	210[a]	249[a]	672[a]	355[a]	161[a]	126[a]	30.0[a]	2.30[b]	62.2[a]
Hytaolive	287[b]	218[a]	1268[b]	566[b]	427[b]	234[b]	41.8[ab]	1.41[a]	118[b]
Sem[2]	10.7	15.2	38.6	20.6	24.3	15.1	3.26	0.066	3.09
P =	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001
Forages									
Alfalfa hay	315	423	1023	625	216	116	55.4	3.03	87.0
Barley straw	250	372	733	463	131	92.2	56.8	3.66	63.0

[a-c] Means in the same row with different superscript differ (P<0.05).

[1] Ac: acetate; Pr: propionate; Bt: butyrate; Minor VFA are calculated as the sum of isobutyrate, isovalerate, valerate and caproate. The amount of organic matter apparently fermented (OMAF) was calculated from VFA production as described by Demeyer (1991).

[2] sem: standard error of the mean.

IV – Conclusions

Crude olive cake samples showed some variations in chemical composition, especially in CP content, but only negligible differences in their *in vitro* fermentation. Fermentation pattern of COC was similar to that of barley straw, but COC showed greater fermentation rates. Crude olive cake could be used in ruminant diets replacing fibrous feeds with low nutritional value, being an interesting alternative due to their high content in unsaturated fatty acids, which may improve the quality of animal products. The two commercial olive extracts tested in this study presented different ruminal fermentation patterns.

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