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Influence of particle size of crude olive cake on *in vitro* ruminal fermentation and gas production kinetics

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Abstract. By-products of olive oil industry can be used in ruminants feeding, but their nutritive value can be affected by the processing method. The aim of this research was to estimate in vitro the nutritive value of four samples of crude olive cake (COC) with different particle size obtained during the drying process from the same trommel at different stages of the screening procedure. Particles size was greater than 3 mm (COC3), 2 mm (COC2) and 1 mm (COC1) or smaller than 1 mm (COC0). Alfalfa hay and barley straw were included in the study for comparative purposes. Chemical composition was analysed in all samples. Gas production kinetics was determined in 144-h in vitro incubations with sheep rumen fluid as inoculum, and fermentation parameters and in vitro dry matter digestibility (IVDMD) were analysed after 24 h of incubation. COC3 and COC0 samples had lower contents of neutral detergent fibre (NDF; 415 and 391 g/kg DM), but greater crude protein (96.6 and 114 g/kg DM) and ether extract (220 and 242 g/kg DM) contents, than COC2 and COC3 (627 and 624; 42.4 and 48.7; 102 and 124 g/kg DM, respectively). IVDMD of COC samples ranged from 39.4 to 58.7%, and was lower than that for alfalfa hay (67.5%) for all samples, but greater than that for barley straw (42.4%) for COC0 and COC3 samples. COC3 and COC0 samples had greater ($P<0.05$) potential gas production (103 and 66.2 ml/g DM, respectively) and total volatile fatty acids production (608 and 600 µmol, respectively) than COC2 and COC1, but values were lower than those for alfalfa hay (202 ml/g DM and 1023 µmol, respectively) and barley straw (198 ml/g DM and 733 µmol). The results indicate that decreasing particle size to <1 mm increased the fermentation potential of COC, but its nutritive value was still slightly lower than that of barley straw. The similar quality observed for COC3 and COC0 samples was attributed to the high sugar content of COC3 samples.

Keywords. Olive cake – Particle size – Chemical composition – Gas production – Ruminal fermentation.

Influence de la taille des particules des grignons d'olive sur la fermentation ruminal et la cinétique de production de gaz

Résumé. *Les sous-produits de l'industrie de l'huile d'olive pourraient être utilisés pour l'alimentation des animaux ruminants, mais sa valeur nutritive peut être affectée par la méthode utilisée pour obtenir ces sous-produits. L'objectif de ce travail a été d'estimer in vitro la valeur nutritive pour des animaux ruminants de quatre échantillons de grignon d'olive (COC) avec différente taille de particules obtenues du même trommel en différents moments du processus de tamisage. La taille des particules était plus grande que 3 mm (COC3), 2 mm (COC2), 1 mm (COC1) et plus Petite que 1 mm (COC0). Foin de luzerne et paille d'orge ont aussi été utilisés pour comparer. La cinétique de la production de gaz a été déterminée en une incubation in vitro (144 heures) avec en utilisant du fluide du rumen de brebis comme inoculum. 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. Les échantillons COC3 et COC1 avaient moins de fibre au détergent neutre (NDF ; 415 et 381 g/kg DM), mais plus des matières azotées totales (96,9 et 114 g/kg DM), et de gras (220 et 242 g/kg DM), que COC2 et COC3 (627 et 624, 42,4 et 48,7, 102 et 124 g /kg DM, respectivement). Les valeurs d'IVDMD des échantillons de COC varié entre 39.4 et 58.7%, étaient plus petite que la valeur du foin de luzerne 67,5%) pour tous les échantillons, mais plus grandes que la valeur de la paille d'orge (42,4%) pour COC0 et COC3. Les échantillons COC3 and COC0 avaient plus (P<0,05) de production potentiel de gaz (103 et 66,2 ml/g DM, respecti-*

vement) et plus des acides gras volatiles (VFA) (608 et 600 µmol, respectivement) que les échantillons COC2 et COC1, mais les valeurs étaient plus petites que les valeurs du foin de luzerne (202 ml/g DM et 1023 µmol, respectivement) et de la paille d'orge (198 ml/g DM and 733 µmol). Les résultats montraient que la réduction de la taille des particules à <1mm augmente la fermentation potentiel, mais la valeur nutritive était encore plus petite que celle de la paille d'orge. La qualité similaire observée pour les échantillons COC3 et COC0 a été attribué à la forte teneur en sucres de l'échantillon COC3.

Mots-clés. *Grignons d'olive – Taille des particules – Production de gaz – Composition chimique – Fermentation ruminal.*

I – Introduction

Olive oil extraction generates several by-products that can be used in animal feeding, especially the cakes and pomaces obtained in the extraction process, but their characteristics and nutritive value depend markedly on the processing method (Feedipedia, 2017). The main product is the "alperujo", which is stored in open ponds until processing, frequently over a period of several months. Alperujo is dried and olive stones are separated from the pulp by a process of screening. This process consists of placing the alperujo in a trommel (a cylinder of perforated metal, open at both ends and with a slight incline), that rotates classifying the material by their particle size (Savage *et al*., 2005). The screening for stones removal produces COC with different particle sizes, and may influence the nutritional quality of COC. The objective of this study was to analyse the influence of particle size of COC on their chemical composition, in vitro ruminal fermentation and gas production kinetics.

II – Material and methods

Four samples of crude olive cake (COC) obtained from the same trommel at different stages of the screening process were used in this study. Samples had different particle size: bigger than 3 (COC3), 2 (COC2) and 1 mm (COC1), or smaller than 1 mm (COC0). Chemical composition was analysed according to the AOAC (2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin analyses were carried out as described by Van Soest *et al*. (1991).

Substrates were fermented in vitro to determine gas production kinetics and ruminal fermentation parameters. One sample of alfalfa hay and barley straw were also incubated for comparative purposes. 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, strained through four layers of cheesecloth 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 CO2. Bottles were prewarmed (39ºC) prior to the addition of 20 ml of buffered rumen fluid, capped and incubated at 39ºC.. Two incubation runs were performed. In the first incubation run, gas production was measured at 3, 6, 9, 12, 18, 24, 36, 48, 72, 96 and 120 h using a pressure transducer (Widereager Wide Range Pressure Meter, Sper Scientific LTD, Scottsdale, AZ, USA) and a calibrated syringe, and the gas produced at each measurement time was released. Bottles without sample (blanks) were incubated to correct for endogenous gas production. 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 CH4 analysis. Bottles were then uncapped and samples for volatile fatty acid (VFA) and NH3-N analyses were taken as described by Martínez *et al*. (2010). Procedures for CH4, VFA and NH3-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, 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 42.4 to 114, 391 to 627, 273 to 408, 122 to150, and 102 to 242 g/kg DM, respectively (Table 1). These values are in the range of those reported by others for COC samples from different sources (Molina-Alcaide *et al*., 2003; Molina-Alcaide and Yáñez-Ruiz, 2008), with the exception of EE content, which was greater in our samples. The high amount of CP bound to the ADF in the COC samples (11 to 43% of total CP) indicated a low N availability, as previously reported for COC (Molina-Alcaide and Yáñez-Ruiz, 2003). The IVDMD of COC samples ranged from 39.4 to 58.7%, and was lower than that for alfalfa hay (67.5%) for all samples, but greater than that for barley straw (42.4%) for COC0 and COC3 samples.

Table 1. Chemical composition (g/Kg dry matter) and in vitro dry matter digestibility (IVDMD) of crude olive cake (COC) samples with different particle size and two forages

	COC3	COC2	COC1	COC0	Alfalfa hay	Straw
Crude protein (CP)	96.6	42.4	48.7	114	194	55.6
Neutral detergent fibre (NDF)	415	627	624	391	394	697
Acid detergent fibre (ADF)	298	408	418	273	215	388
Lignin	122	148	163	150	45.0	38.8
Crude protein bound to the ADF	29.4	10.7	12.7	42.5	66.2	19.2
Ether extract	220	102	124	242	47.2	28.5
IVDMD (%)	58.7	39.4	40.9	49.4	67.5	42.4

As shown in Table 2, COC3 had the greatest ($P<0.05$) asymptotic gas production (A) and COC2 and CO1 had the lowest values. The higher gas production values observed in COC3 would indicate a more extensive fermentation, as cumulative gas production is directly related with the amount of organic matter fermented (Menke *et al*., 1979). In contrast, there were no differences ($P \geq 0.146$) among COC samples for c and lag parameters. COC3 also showed a greater value ($P<0.05$) of AGPR compared with the other samples. Cumulative gas production over the first 24 h of incubation was greater for COC3 and COC0 than that for barley straw, but COC2 and COC1 showed similar values to straw (Figure 1). Gas production for all COC samples was lower than that for alfalfa hay at all sampling times.

Table 2. Gas production parameters of crude olive cake (COC) samples with different particle size and two forages (n = 4)[1]

Substrate[1]	A	c	*lag*	AGPR
COC3	103[c]	0.0558	0.40	3.98[b]
COC2	29.1[a]	0.0801	2.08	1.37[a]
COC1	30.7[a]	0.0813	1.76	1.54[a]
COC0	66.2[b]	0.0531	1.21	2.35[a]
sem[2]	2.14	0.01002	0.230	0.230
P =	<0.001	0.146	0.242	<0.001
Forages				
Alfalfa hay	202	0.0442	1.82	5.73
Barley straw	198	0.0303	15.4	2.57

[a-c] Means in the same row 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.

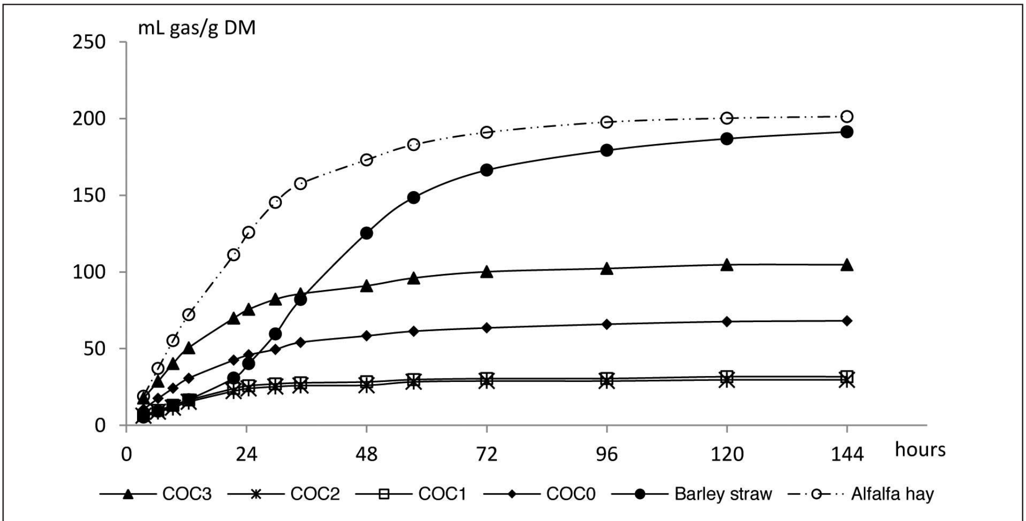

Fig. 1. *In vitro* cumulative gas production of crude olive cake (COC) samples with different particle size and two forages.

The results of the 24 h in vitro fermentations (Table 3) were in well agreement with those observed for gas production kinetics. Both COC3 and COC0 produced more (P<0.05) total VFA, acetate, propionate and CH4 than COC2 and COC1, resulting in greater (P<0.05) amount of OMAF. In contrast, there were no differences (P>0.05) among COC in NH3 concentrations, which may be due to the use of a N-enriched buffer solution. The greater fermentation of COC0 may be explained by its lower stone content, as stones are low fermentable. The high fermentation observed in COC3 may have been due to a high sugar content, as this sample contained small green-coloured balls which were identified as caramelized sugars (Bacha, personal communication). Both VFA and CH4 production were lower in all COC samples than in the two tested forages, indicating that COC samples were less fermented.

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

Substrate	CH_4 (µmol)	NH_3-N (mg/L)	Total VFA (µmol)	(µmol) Ac	Pr	Bt	Minor VFA	Ac/Pr (mol/mol)	OMAF (mg)
COC3	190^b	348	628^b	352^b	141^b	90.2^b	45.5^b	2.59^a	54.5^b
COC2	144^a	382	406^a	226^a	73.4^a	65.8^a	41.0^a	3.15^b	34.9^a
COC1	155^a	366	451^a	249^a	83.5^a	73.4^{ab}	45.4^b	3.07^b	38.8^a
COC0	187^b	349	600^b	338^b	131^b	84.3^{ab}	47.3^b	2.68^a	51.6^b
sem2	6.10	11.2	14.5	7.50	4.85	4.47	3.62	0.052	1.44
P =	<0.001	0.167	<0.001	<0.001	<0.001	0.016	0.007	<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] 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

Olive cake samples differing in particle size showed marked differences in chemical composition and in vitro ruminal fermentation parameters, with samples having more than 3 mm or less than 1 mm showing the highest fermentability. Crude olive cake could be used in ruminant diets replacing fibrous feeds with low nutritional value, but due to its high fat content olive cake would also contribute to increase the energy level of the diet.

Acknowledgments

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References

AOAC. 2000. Official Methods of Analysis, Editorial Association of Official Analytical Chemists. (17th ed., 6th rev.), Internacional.

Demeyer D.I., 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. In: *Rumen Microbial Metabolism and Ruminant Digestion*, ed. Jouany JP. INRA Editions, Paris, France, p. 217-237.

Feedipedia, 2017. Olive oil cake and by products 2017. Feedipedia Homepage www.feedipedia.org. Download at http:// http://www.feedipedia.org/node/32. (Accessed on 14/06/2017).

Goering H.K., Van Soest P.J., 1970. Agricultural Research Service-USDA, Washington, D.C.

Martinez M.E., Ranilla M.J., Tejido M.L., Ramos S., Carro M.D., 2010. The effect of the diet fed to donor sheep on in vitro methane production and ruminal fermentation of diets of variable composition. In: *Anim. Feed Sci. Technol*., 158, p. 126-135.

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor in vitro. In: *J. Agr. Sci.*, 93, p. 217-222.

Molina Alcaide E., Yáñez Ruiz D.R., Moumen A., Martín García I., 2003. Chemical composition and nitrogen availability of some olive by-products. In: *Small Ruminant. Res*., 49, p. 329-336.

Molina Alcaide E., Yáñez-Rúiz D.R., 2008. Potential use of olive by-products in ruminant feeding. In: *Anim. Feed Sci. Tech*., 147, p. 247-264.
Savage G., Díaz L., Goldstein N., 2005. A compost screening premier. In: *BioCycle*, ed. J.G. Press Inc, Emmaus, United States; 46, 5, p. 55-59.
Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. In: *J. Dairy Sci.*, 74, p. 3583-3597.