Nutritional value of crude and partially defatted olive cake in finishing pigs and effects on nitrogen balance and gaseous emissions

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Abstract

By-products from the food industry can be valuable ingredients in animal feeds. One example is olive cake (OC), generated in large amounts by the olive oil industry, which contains oil with a high proportion of oleic acid and polyphenols. An experiment was performed using pigs to determine the nutritional value of crude (COC) and partially defatted (PDOC) olive cake, and to evaluate their effect on nutrient balance, slurry properties and potential ammonia (NH_3) and methane (CH_4) emissions. Five experimental feeds were designed; a basal diet and another four diets produced by substituting 100 or 200 g/kg of the basal diet with either COC or PDOC. Thirty finishing male pigs (76.1 ± 4.2 kg initial BW) were used in the experiment (6 animals/treatment). After a 14-day adaptation period, faeces and urine were collected separately for 7 days to measure nutrient digestibility and the excretory patterns of nitrogen. Potential NH_3 and CH_4 emissions were measured in reconstituted slurry samples over 11 and 100 days, respectively. The dry matter (DM), crude protein (CP), cellulose, starch and energy coefficients of total tract apparent digestibility (CTTAD) were negative and linearly ($P<0.05$) affected by OC inclusion level. However, the type of OC did not influence any of the digestion efficiencies studied. The energy digestibility of the ingredients tested, estimated by substitution, were 0.479 (±0.040, SEM) and 0.327 (±0.049) for COC and PDOC, respectively. Faecal content of cellulose, polyphenols and gross energy (GE) increased linearly with OC inclusion, whereas ash content decreased. The total N content of urine decreased linearly with OC inclusion, but benzoic and hippuric acid contents increased, which resulted in lower pH values for the OC diets. The ratio between faecal and urine N excretion decreased from 2.48 in the basal diet to 1.01 on average in the 200 g/kg OC diets. As a result, increasing both COC and PDOC levels in diets resulted in lower NH_3 emissions per volume of slurry and in a lower biochemical CH_4 potential. Although slurry excretion increased with OC inclusion, daily NH_3 emissions still decreased with increasing OC inclusion. However, potential CH_4 emissions per

animal increased. Overall, the results indicate a digestible energy (DE) values from OC and COC that account for about 59-79% of the DE provided by the barley, respectively. A global perspective is needed to assess the impact of including OC in pig diets throughout the production chain.

Keywords: ammonia emission, digestion efficiency, methane emission, nitrogen balance, olive cake

Abbreviations: ADFom, acid detergent fibre without residual ash; ADL, acid detergent lignin; AOAC, Association of Official Analytical Chemists; BMP, biochemical methane potential; CH_4, methane; COC, crude olive cake; CTTAD: coefficient of total tract apparent digestibility; DM, dry matter; CP, crude protein; DE, digestible energy; GE, gross energy; aNDFom, neutral detergent fibre; NDICP, neutral detergent insoluble crude protein; NH_3, ammonia; OC, olive cake; OM, organic matter; PDOC, partially defatted olive cake; SEM, standard error of means; SF, soluble fibre; TAN, total ammonia nitrogen; TDF, total dietary fibre; TKN, total Kjeldahl Nitrogen; VFA, volatile fatty acids.

1. Introduction

Worldwide pork production reached 115 Tg in 2014 (Food and Agriculture Organization Corporate Statistical Database FAOSTAT, 2017), and is expected to rise in the coming decades (Alexandratos and Bruinsma, 2012). However, increasing intensification of pig production raises concerns about its sustainability, especially in terms of nutrient use. Nearly two thirds of the EU's cereals are used in animal feeds (European Commission, 2017), but, on average, only 25-30% of global animal dietary calories are retained in meat and milk products. Consequently, a relevant proportion of nitrogen and organic matter intake is excreted, which has important potential environmental impacts. According to the European Environment Agency (2017a and 2017b), pig slurries in the EU are responsible for about 15% of ammonia (NH_3) and 4% of total methane (CH_4) emissions. In the future, the feed industry will need to find alternative feedstuffs and minimize their eco-footprints.

By-products from the food industry often constitute a serious management problem both in economic and environmental terms, but they also involve great losses of valuable nutrients (Mirabella et al., 2014). Olive oil production is a major industry in Mediterranean countries and generates large amounts of waste. Olive cakes (OC) consist of a mixture of olive pulp, skin, stone, water and residual oil, in variable proportions, which are often dried to facilitate further use (Uribe et al., 2013). Although OC is widely used as biofuel (Casanova-Peláez et al., 2015), its nutrient content and phenolic makeup may be useful for animal nutrition (Uribe et al., 2013).

Oil extraction procedures affect the amount and composition of olive by-products (Vlyssides et al., 1998; de Blas et al., 2015). Crude OC has an appreciable oil content with a high proportion of oleic acid (Joven et al., 2014). When cost-efficient, it can be partially or totally extracted, based on variability in the market price of olive oil. The by-products are generally dried and available throughout the year. The OC can potentially be used as a source

of energy in diets for finishing pigs and sows, which would contribute to circular economy in Mediterranean countries such as Spain, the most important olive oil producer and the fourth biggest pig producer worldwide (FAOSTAT, 2017). Previous research (Beccaccia et al., 2015a) has shown that including fibrous by-products (such as orange pulp or carob meal) in diets for growing pigs can help to reduce ammonia and methane emissions from slurry, per unit of nitrogen (N) or organic matter (OM), respectively. The objective of this study was to determine the nutritive value for fattening pigs of crude (COC) and partially defatted (PDOC) olive cake and evaluate their effects on nutrient balance, slurry composition and gaseous emissions from slurry, when included in pig diets.

2. Material and methods

2.1. Animals, diets and experimental design

The experimental procedure was approved by the Ethics Committee of the Universitat Politècnica de València (registration number 2016/VSC/PEA/00024). Thirty finishing male pigs, progeny of Pietrain x (Landrace x Large White) with 76.1±4.2 kg initial BW were used in the experiment (6 animals per treatment) in three batches of 10 animals each. Crude and partially defatted OC were provided by a local processor (DCOOP, Antequera, Spain (see Table 1 for their chemical composition). Five experimental feeds were designed; a basal diet and another four diets produced by substituting 100 or 200 g/kg of the basal diet with either COC or PDOC. The basal diet was formulated with common ingredients (barley, corn, wheat, and soybean meal) in commercial feeds to meet or exceed net energy, protein and essential amino acid levels in order to avoid an excessive imbalance in diets supplemented with OC with respect to the recommendations of the *Fundación Española para el Desarrollo de la Nutrición Animal* (FEDNA, 2013) for fattening pigs (20-100 kg). Ether extract (EE) and fibre contents were kept low in the basal diet to compensate for the high content of these components in OC.

111 The ingredients and chemical composition of the experimental diets are shown in Tables 2 and
112 3, respectively.
113 The experimental period consisted of a 14-day adaptation period to diets, followed by 7
114 consecutive days during which faeces and urine were collected individually, as described in
115 Beccaccia et al. (2015a). At the beginning of the experiment, pigs were assigned to one of five
116 dietary treatments and placed in conventional pens until day 9 of the adaptation period. After
117 that, they were housed individually in metabolism pens (1.2x2 m^2) until the end of the
118 experiment, where individual feed intake could be measured as well as total faeces and urine
119 (collected separately). The collection period was divided in two parts to facilitate collections
120 for energy and nutrient balance (days 1-4) and gaseous emissions (days 5-7). Feed and water
121 were provided *ad libitum* throughout the experimental period. Feed was provided in dry form
122 (pelleted). Pigs were weighed individually at the beginning of the adaptation period.
123
124 *2.2.Experimental procedures and sample preparation*
125 The experimental procedures and sample preparation for the energy and nutrient balance
126 and emissions period followed the procedure described in Beccaccia et al. (2015a). Briefly,
127 during the energy and nutrient balance (4 days), feed consumption was measured and total
128 urine and faeces excreted per animal were collected daily in separate buckets, weighed and
129 stored in a chamber at 4ºC until the end of the collection period. Urine was collected under
130 sulphuric acid (120 mL of H_2SO_4 at 10% per bucket and day). Upon final collection the faeces
131 and urine were pooled per pig, mixed, subsampled and stored at -20ºC until laboratory analyses
132 were performed. During the next 3 days (days 5-7), urine and faeces were collected in a similar
133 way, but without any addition of sulphuric acid to urine. On day 7, slurries were reconstituted
134 by mixing urine and faeces from each animal in the same proportion as excreted. A part of
135 these slurries was used in fresh for pH and NH_3 emission measurements and another one was

136 subsampled and frozen (-20ºC) for determination of slurry characteristics and biochemical
137 methane potential (BMP).
138
139 *2.3. Chemical analysis of feeds and effluents*
140 Feeds and faeces from the nutrient balance period were analysed for DM (930.15), ash
141 (923.03), EE (920.39) and total dietary fibre (985.29) according to the Association of Official
142 Analytical Chemists (AOAC, 2000) procedures. The concentrations of neutral detergent fibre
143 (aNDFom), acid detergent fibre (ADFom) and acid detergent lignin (ADL) were determined
144 sequentially using the filter bag system (Ankom Technology Corp., Macedon, NY, USA)
145 according to Mertens (2002), AOAC (2000; procedure 973.187) and Van Soest et al. (1991),
146 using heat stable amylase (FAA, Ankom Technology Corp., Macedon, NY, USA), and
147 expressed without residual ash. The contents in soluble fibre were estimated from the
148 difference between total dietary fibre and aNDFom corrected by CP content in the residue. The
149 contents in hemicelluloses and cellulose were estimated, respectively, from the differences
150 between aNDFom and ADFom and ADFom-ADL concentrations. Feed and faeces were
151 defatted with petroleum ether prior to fibre analysis. The gross energy (GE) concentration was
152 measured in an isoperibol bomb calorimeter (Parr 6400, Parr Instruments Co., Moline, IL,
153 USA). Total N was measured by combustion (method 986.06; AOAC, 2000) using Leco
154 equipment (model FP-528, Leco Corporation, St. Joseph, MI, USA) and CP estimated as N
155 content x 6.25. The proportion of neutral and acid detergent insoluble CP in feed and faeces
156 samples was determined following the standardized procedures in Licitra et al. (1996). The
157 polyphenolic compounds present in OC, diets and faeces were determined after extraction with
158 methanol/acetone/water following the procedure described by Chamorro et al. (2012).
159 Urine was freeze-dried to obtain its DM content and mixed with benzoic acid before GE
160 analysis to make sure that the whole sample was burned. Total N was determined by steam

161 distillation (APHA, 2005) using an automatic analyser (2300 Kjeltec, Foss Analytical,
162 Hilleroed, Denmark). Hippuric and benzoic acids were analysed directly in urine samples via
163 high performance liquid chromatography (HPLC) on a Varian Pro Star 310 HPLC system
164 (Varian Inc., Palo Alto, CA, USA) following the procedure described by Sánchez-Martín et al.
165 (2017).

166 Additionally, the pH of faeces, urine and slurry was measured in duplicate using a glass
167 electrode (Crison Basic 20+, Crison, Barcelona, Spain). The pH of faeces was determined by
168 mixing samples with deionized water at a 1:1 proportion. Slurry pH was measured immediately
169 after reconstitution. Slurry samples were analysed for DM and ash following the same
170 methodology used for faeces analyses, and total ammonia N (TAN) and total Kjeldahl N (TKN)
171 using that for urine samples. To avoid N volatilization, the subsample used for TAN analyses
172 was acidified with HCl immediately after reconstitution. Volatile fatty acids (VFA)
173 concentration was determined by gas chromatography equipped with a flame ionization
174 detector (HP 68050 series Hewlett Packard, USA) following the method described by Jouany
175 (1982) with the addition of an internal standard (4-metil valeric).

176

177 *2.4. Gaseous emissions monitoring*

178 The procedure for gas emission monitoring was previously described in Beccaccia et al.
179 (2015a). Briefly, two replicate slurry samples of 0.5 kg from each animal were placed in a 1 L
180 closed container maintained at 25ºC in a thermostatic water bath (Selecta, Barcelona, Spain),
181 and 50 mL of distilled water was added to prevent surface crust formation in each container.
182 Containers were used as dynamic chambers and were connected to an air pump which extracted
183 air at a constant airflow rate of 1.2 L/min. During 11 consecutive days, the air was forced to
184 pass through 2 absorption flasks (impingers) in serial containing 100 mL of sulphuric acid 0.1
185 N. The acid solution was replaced daily during the first 5 days, and every 48 h until the end of

186 the assay (day 11). The NH_3 trapped in the impingers was analysed following 4500 NH_3-D
187 procedure (APHA, 2005) using a detection electrode (Orion High Performance NH_3 Electrode,
188 model 9512HPBNWP, Thermo Scientific, Waltham, MA, USA).
189 Biochemical CH_4 potential from slurry was measured as the cumulative CH_4 production
190 per gram of OM in a batch assay, using 120 mL glass bottles incubated at a mesophilic range
191 (35°C±1ºC) for 100 days, following the methodology described by Angelidaki et al. (2009).
192 Anaerobic inoculum was collected from an anaerobic digester that treated pig slurry, and pre-
193 incubated during 15 days at 35ºC in order to deplete the residual biodegradable organic material
194 (degasification). An inoculum to substrate ratio of 1 on OM basis was used. Slurry samples
195 from each animal were tested by triplicate and three blank bottles containing only anaerobic
196 inoculum were used in order to determine its endogenous CH_4 production. After filling, each
197 bottle was sealed with butyl rubber stoppers and aluminium crimps and the headspace was
198 flushed with pure N_2 for two minutes. During incubation, biogas volume in each bottle was
199 regularly monitored (from 1 to 10 days depending on biogas production) by pressure
200 measurement of the headspace using a manometer (Delta Ohm, HD 9220, Padova, Italy).
201 Methane concentration in the biogas was further analysed using a Focus Gas Chromatograph
202 (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector.
203 *2.5. Calculations and statistical analysis*
204 The apparent total tract digestibility (CTTAD) of energy, CP, aNDFom and EE of the two
205 OCs studied was determined by difference, assuming additivity between the basal diet and the
206 ingredients tested in the experimental diets. Calculations were made by correcting the CTTAD
207 of energy at the 200 g/kg level of inclusion of OC with the energy contribution of the basal
208 diet, as described in Bolarinwa and Adeola (2012). The CTTAD of energy was also determined
209 by extrapolation to a total substitution (100%) using linear regression (REG procedure of SAS,
210 2008) between the CTTAD of energy and the proportion of basal diet substituted with OC at

211 the100 and 200 g/kg levels of inclusion. The digestible energy (DE) content of OC was
212 estimated from the product between the estimates of CTTAD of energy and its GE
213 concentration (Table 1). Each animal was the experimental unit for all the traits studied. The
214 whole data set derived from the five dietary treatments was analysed in a two factors ANOVA
215 as a completely randomized block design, with diet as the main effect and batch as a block
216 factor. The effects of diet were analysed using specific contrasts to test for the effects of type
217 and level of inclusion of OC and of its interaction. The effects of increasing levels of OC were
218 tested using linear and quadratic orthogonal contrasts (Steel et al., 1997).
219
220 **3. Results**
221 *3.1. Coefficients of total tract apparent digestibility*
222 The influence of trial batch and of its interactions with dietary treatments was not
223 significant for any of the traits studied (data not shown). The CTTAD of the different nutrients
224 analysed in the experimental diets is presented in Table 4. The values for DM, cellulose, starch
225 and GE were negative and linearly ($P<0.05$) affected by the inclusion level of OC. An
226 interaction ($P=0.024$) between type and level of OC was observed for CTTAD of CP, as a
227 greater decrease (0.084) was observed in the case of PDOC than for COC (0.028). Moreover,
228 energy losses in urine, expressed as a proportion of DE, increased linearly with level of
229 inclusion for both types of OC (from 0.028 in the control diet to an average of 0.046 in the
230 diets containing 200 g/kg of OC).
231 Ether extract CTTAD was quadratically affected ($P<0.001$) by dietary OC addition, with
232 higher increments being observed at 100 g/kg in the basal diet than from 100 to 200 g/kg.
233 Otherwise, the total polyphenols CTTAD increased linearly ($P<0.001$) with dietary OC level.
234 Type of OC did not influence any of the digestion efficiencies studied. A significant interaction

between the level and type of OC was observed for CP, as the decrease in CTTAD with the inclusion of OC was greater in the case of PDOC than with COC.

The CTTAD of energy of the ingredient tested was determined by the difference method at the 200 g/kg level of inclusion of OC. The results obtained were 0.479 (±0.040, SEM) and 0.327 (±0.049) for COC and PDOC, respectively. Using the GE content shown in Table 1, the DE concentrations of COC and PDOC were estimated, respectively, to be 11.2 (± 0.89) and 7.40 (±1.11) MJ/kg DM. The same method led to higher ($P<0.05$) CTTAD for COC than for PDOC in the case of CP (0.375±0.046 vs 0.173±0.060) and EE (2.05±0.094 vs 1.78±0.095). The value obtained for aNDFom in COC was also higher than for PDOC (0.340±0.215 vs 0.282±0.209), although in this case the difference did not reach statistical significance. The CTTAD of energy of the OC studied was also estimated from regression equations between the values obtained for the experimental diets and the substitution levels of OC (S). The equations obtained were:

CTTAD of energy_COC=0.871 (±0.0069) - 0.397 (±0.053) S; $P<0.001$; RSD=0.020; R^2=0.745.

CTTAD of energy_PDOC=0.878 (±0.0080) - 0.549 (±0.062) S; $P<0.001$; RSD=0.023; R^2=0.805.

dCP_COC= 0.847 (±0.0091) - 0.478 (±0.071) S; $P<0.001$; RSD=0.026; R^2=0.706.

dCP_PDOC=0.859 (±0.012) - 0.680 (±0.093) S; $P<0.001$; RSD= 0.035; R^2=0.736.

Extrapolating these equations to S=1, the CTTAD of energy of COC and PDOC was estimated to be 0.474 (± 0.90, SEM) and 0.329 (± 0.92).

3.2. Composition of effluents

The effects of type of diet on the composition of faeces and urine are shown in Table 5. Inclusion of OC in the diet did not modify starch or aNDFom contents in faeces (averaging

50.4 and 473 g/kg DM respectively), but linearly increased those of ADFom, ADL, hemicelluloses and cellulose, polyphenols and GE concentration. On the contrary, ash faecal content decreased linearly and that of EE linearly and quadratically with dietary OC level. Urine composition was also affected by type of diet, as adding OC led to a linear decrease in TKN (by 35.8% at the highest levels of inclusion), whereas benzoic acid, hippuric acid and GE concentration all increased (by 96, 146 and 33%, respectively, comparing controls and the average from 200 g/kg OC). Urine pH decreased linearly (P=0.047) with level of OC, from 8.20 in the control diet to 7.74 on average for diets with the highest levels of OC. Neither source nor the interaction level x source of OC affected any of the effluent composition traits studied.

3.3. Dry matter and nitrogen flows

Type of diet did not affect DM intake expressed per $kg^{0.75}$ (Table 6). However, inclusion of OC led to a linear increase (P<0.001) in faecal DM excretion, with values tending (P=0.07) to be higher as average for PDOC than for COC diets (16.9 vs 12.1 $g/kg^{0.75}$, respectively), and a trend (P=0.09) for a higher urine DM excretion (by 25.9% on average at the highest OC levels with respect to the control diet). In the case of N balance, no differences among treatments were observed either in N intake or N retention (that averaged 2.04 and 0.99 $g/kg^{0.75}$, respectively). However, adding any OC in diets linearly increased N excretion in faeces and decreased it in urine. Consequently, the ratio between faecal and urine N excretion decreased from 2.48 in the basal diet to 1.01 on average in the 200 g/kg OC diets. No differences were observed for DM or N balance between OC sources or for the interaction among sources and levels of inclusion.

3.4. Slurry characteristics and gaseous emissions

Table 7 shows the effects of including different levels of COC and PDOC in the diets on slurry (faeces+urine) excretion, composition and emissions. The inclusion of either COC or PDOC increased fresh slurry excretion. Additionally, both the DM and OM content of slurry increased, by approximately 18 and 24% per each 10% increase of OC in diet, respectively. On the contrary, a linear reduction of TKN content was observed, mainly because of a reduction in the total ammonia N content. An interaction (P=0.04) of type and level of OC was observed for slurry pH, which decreased greatly for COC than for PDOC. The concentration and profile of VFA was not affected by the inclusion of either COC or PDOC. There was no effect of OC type on any of the slurry properties. The inclusion of OC also reduced NH_3 emissions per kg slurry by more than 40% for the highest inclusion rates of both COC and PDOC. Despite the higher slurry excretion rates of pigs fed OC diets, NH_3 emissions also tended to decrease when they were expressed per animal and on a daily basis. In terms of available TKN, the emissions of NH_3 were also lower for COC and PDOC diets compared with the basal diet. Biochemical methane potential (mL CH_4/g OM) decreased linearly (P=0.05) with OC inclusion, but as slurry excretion and OM content in slurries increased with the inclusion of OC, higher CH_4 emissions were estimated (with OC) when expressed as L of CH_4 per day.

4. Discussion

4.1. Nutritional value of olive cake sources

The CTTAD of energy and DE values determined either by difference or by regression (extrapolation to S=1) were similar for both OC samples, but the SE of the estimations was much lower when they were calculated by difference. The determined energy values were higher for COC than for PDOC, mostly due to a higher EE content (0.162 vs. 0.119, on an as fed basis). The CTTAD of energy for COC (0.479) was close to that assigned (0.446) by Heuzé et al. (2015) for a COC with a similar EE content to that used in the current study (171 g/kg

309 DM). Otherwise, the increase in energy losses in urine in both OC might be related to the higher
310 excretion of benzoic and hippuric acids. Overall, the results indicate a limited energy value for
311 this ingredient, especially for PDOC, which fits well with the decrease (12%) of feed efficiency
312 observed by Joven et al. (2014) when replacing directly 150 g/kg of barley with COC in the
313 diet of growing pigs.

314 Estimations of CTTAD for EE in both OC were higher than 1. This result may be explained
315 by a decrease in the relative contribution of endogenous lipids to total faecal output with OC
316 addition, which led to a linear, but also quadratic, increase in the dietary CTTAD of EE. Also,
317 the fatty acid profile of OC is characterized by a high proportion (0.679) of highly digestible
318 oleic acid (Joven et al., 2014). The relatively low CTTAD of CP (mainly in the case of PDOC)
319 might be related to the high proportion of insoluble CP in aNDFom (0.672 and 0.902 in COC
320 and PDOC, respectively) and in ADFom (0.351 and 0.476). This low efficiency together with
321 a relatively low CP concentration in the ingredients tested implied that the amount of digestible
322 CP provided by COC and PDOC (36.0 and 13.8 g/kg, respectively) was quite lower than the
323 present recommendations in this species. The high degree of lignification of aNDFom (0.348
324 as average of both OC) would explain the low CTTAD of aNDFom observed. Otherwise, the
325 appreciable supply of insoluble fibre with this ingredient can be valuable to meet the current
326 allowances of aNDFom in pig diets, especially in the case of pregnant and lactating sows and
327 in growing pigs (180, 150 and 110 g/kg respectively, according to FEDNA, 2013).

328

329 *4.2. Nutrient balance and effluent composition*

330 The inclusion of OC affected the composition of effluents and the N balance. The intake of
331 DM and N did not change among treatments, but the inclusion of OC affected the amount
332 excreted and the composition of faeces and urine, which had a direct impact on NH_3 emission.
333 Increasing the levels of OC increased total DM and N excretion in faeces, mainly because of

334 the decreased DM and N CTTAD. On the contrary, the N concentration and total excretion of
335 urine N decreased with increasing OC inclusion rates. As a result, the urine:faecal N ratio
336 decreased from 2.48 in the control diet to 1.01 in the diets containing 200 g/kg of either COC
337 or PDOC. This ratio has been proposed as a main indicator of NH_3 emission, and the reported
338 values in the literature range between 1.2 and 3.8 (Canh et al., 1997 and 1998).
339 Although there were no significant changes in slurry VFA, both benzoic and hippuric acid
340 contents in urine increased with increasing OC inclusion levels. In several species, benzoic
341 acid and its metabolite hippuric acid have been described as secondary metabolites when
342 animals are fed polyphenol-rich diets (Gonthier et al., 2003). The current results show that the
343 total excretion of benzoic and hippuric acids ranged from 1.05 to 2.38 mg/mL in the control
344 and 200 g/kg OC diets, respectively. That increment was associated with increasing levels of
345 intake, absorption and excretion of polyphenols and fibre. Those values are within the range of
346 values reported in the literature for growing pigs using fibrous ingredients (Sánchez-Martín et
347 al., 2017), but are lower than in ruminants, where hippuric acid accounts for about 5% of the
348 N excreted (Bristow et al., 1992, Bussink and Oenema, 1998). Recent studies have
349 demonstrated that both benzoic and hippuric acids may inhibit nitrification in soils, and
350 therefore reduce nitrous oxide emissions from slurry applied to soil (Kool et al., 2006; Sánchez-
351 Martín et al., 2017), which seems to be related with changes in pH and the corresponding
352 reduction of NH_3 emission. Benzoic acid has been tested as a feed additive to reduce NH_3
353 emissions. Adding 1% benzoic acid to the diet reduces urine pH by about 1 unit, and increases
354 urinary hippuric acid content to more than 10 mg/L, compared to about 1 mg/L in control diets
355 (Bühler et al., 2006). In the current study, slurry pH decreased in parallel with an increase of
356 benzoic+hippuric acid associated with OC supplementation. That finding, along with the lower
357 urine:faecal N ratio and the lower ammonia N concentration in the slurry, would help to explain
358 the decrease (by 28%) of NH_3 emission per kg of slurry when adding 200 g/kg of OC. The

359 decrease was lower (by 20.4%) when expressed per animal and day, because of the parallel
360 increment in the amount of slurry excretion.

361 Biochemical methane potential expressed as mL CH_4/g OM decreased with OC
362 inclusion. Although higher BMP values were reported when supplementing diets with low
363 digestible fat sources (Antezana et al., 2015), the CTTAD of EE of the ingredients used in the
364 current study was high, so the faecal concentration of EE decreased (by 31% on average) with
365 dietary addition of 200 g OC/kg. Moreover, faecal ADL content increased (by 80% on average)
366 with OC inclusion, whereas faecal concentration of aNDFom did not vary with type of diet.
367 Previous studies (Angelidaki et al., 2009; Triolo et al., 2011and Beccaccia et al., 2015b) have
368 shown that ADL concentration in OM is negatively correlated with BMP. Polyphenols have
369 been reported to potentially inhibit methanogenesis, but the content of polyphenols in the
370 faeces (ranging from 99 g/kg DM for the basal diet to 274 g/kg DM in the 200 g/kg inclusion
371 of PDOC) were lower than levels found by Akassou et al. (2010) as potentially causing
372 inhibitions.

373 When taking into account the higher slurry excretion in OC diets, as well as the higher
374 OM content in those slurries, the overall effect of OC inclusion would be to increase BMP
375 (around 100%), when expressed per animal and per day. Thus, it is necessary to evaluate
376 whether the potential increase in CH_4 emissions from the slurry may be compensated by the
377 potential mitigation of greenhouse gas emissions gained by using industrial by-products instead
378 of crops, and considering the potential implications on animal performance.

379 **5. Conclusions**

380 The nutritional value of the OC's tested indicate a limited energy value for these
381 ingredients, especially for PDOC, a low CTTAD of CP and an appreciable supply of insoluble
382 fibre. Thus, the use of OC in pig diets can be valuable to meet the current allowances of

aNDFom, especially in the case of pregnant and lactating sows and in growing pigs but limiting in the CP and DE provided.

Regarding effluents composition, the inclusion of OC increased total DM and N excretion in faeces whereas leads to lower N excretion in urine and lower pH values from slurry with an increase of benzoic+hippuric acid content. Associated to pig slurry modifications, NH_3 emission and BMP per kg of slurry decreased with the inclusion of OC. Nevertheless, slurry excretion increased with OC inclusion, leading to higher BMP when expressed per animal and per day. Although these results indicate a potential for increased CH_4 emissions, a global perspective is needed based on carbon footprint analysis to assess the impact over the whole production chain.

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499 **Table 1** *Chemical composition of the olive cakes (OC) studied (g/kg, as fed basis)*

500

	Crude OC	Partially defatted OC
Dry matter	932	937
Ash	83.4	89.5
HCl insoluble ash	6.66	6.37
Crude protein	96.0	79.9
NDICP[a]	59.7	72.1
ADICP[b]	33.7	38.0
Ether extract	162	119
aNDFom	356	367
ADFom	227	247
ADL	121	131
Total polyphenols	15.9	19.8
Sugars	97.0	100
Gross energy (MJ)	21.7	21.2

501 [a]Neutral detergent insoluble CP.
502 [b]Acid detergent insoluble CP
503

504 **Table 2** *Ingredient composition of the experimental diets (g/kg, as fed basis)*

	Diets[a]				
	Basal	100_COC	200_COC	100_PDOC	200_PDOC
Barley grain	300	270	240	270	240
Corn grain	300	270	240	270	240
Wheat grain	163	147	131	147	131
Soybean meal 48	205	184	164	184	164
Crude olive cake	0	100	200	0	0
Partially defatted olive cake	0	0	0	100	200
Calcium carbonate	7	6.3	5.6	6.3	5.6
Dicalcium phosphate	12.8	11.5	10.2	11.5	10.2
Sodium chloride	4.2	3.8	3.4	3.8	3.4
DL-methionine	0.4	0.4	0.3	0.4	0.3
L-lysine HCL	2.3	2.1	1.9	2.1	1.9
L-threonine	0.5	0.5	0.4	0.5	0.4
Premix[b]	5	4.5	4	4.5	4

505 [a]100_COC = 100g/kg crude olive cake; 200_COC = 200g/kg crude olive cake; 100_PDOC = 100g/kg
506 partially defatted olive cake; 200_PDOC = 200g/kg partially defatted olive cake.
507 [b]Vitamin and mineral premix supplied per kg complete diet: 5000 IU of vitamin A; 1000 IU of vitamin D3;
508 3 mg of vitamin B2; 20 mg of vitamin B12; 10 mg of niacin; 4 mg of pantothenic acid; 48 mg of betaine;
509 30 mg of manganese oxide; 110 mg of zinc oxide; 10 mg of copper sulphate; 0.75 mg of potassium iodide;
510 0.1 mg sodium selenite; 90 mg of iron carbonate.
511

512 **Table 3** *Chemical composition of the experimental diets (g/kg, as fed basis)*

	Diets[a]				
	Basal	100_COC	200_COC	100_PDOC	200_PDOC
Dry matter	896	897	898	895	898
Ash	50.4	56.4	61.0	54.8	59.2
Crude protein	176	169	161	172	156
Ether extract	16.2	28.4	39.7	26.7	34.9
aNDFom	110	130	155	139	164
ADFom	42.5	61.6	85.0	67.5	89.8
ADL	8.1	19.1	33.7	23.9	39.1
Total polyphenols	0.36	1.44	3.15	1.41	3.11
Starch	434	408	350	383	361
Sugars	72.5	66.9	63.5	73.8	73.5
Calcium[b]	6.7	7.0	7.4	7.0	7.4
Phosphorous	5.8	5.4	5.0	5.4	5.0
Gross energy (MJ/kg)	16.4	16.7	17.2	16.7	17.1
Net energy (MJ/kg)[b]	9.7	9.4	9.0	9.0	8.4
Ileal standardized digestible amino acids[b]					
Lysine	9.1	8.2	7.3	8.2	7.3
Methionine	2.8	2.5	2.2	2.5	2.2
Total sulphur	5.4	4.8	4.3	4.8	4.3
Threonine	5.8	5.2	4.6	5.2	4.6
Tryptophan	1.8	1.6	1.4	1.6	1.4
Isoleucine	6.1	5.5	4.9	5.5	4.9
Valine	7.1	6.4	5.7	6.4	5.7

513 [a]100_COC = 100g/kg crude olive cake; 200_COC = 200g/kg crude olive cake; 100_PDOC = 100g/kg
514 partially defatted olive cake; 200_PDOC = 200g/kg partially defatted olive cake.
515 [b]Values calculated according to FEDNA (2010).

Table 4 *Effect of type and level of inclusion of olive cake (OC) on the apparent total tract digestibility coefficients and energy balance of the experimental diets*

	Diets[a]						Significance[b]			
	Basal	100_COC	200_COC	100_PDOC	200_PDOC	SEM[c]	1	2	3	4
Dry matter	0.875	0.826	0.808	0.831	0.781	0.0081	<0.001	0.44	0.17	0.054
Ash	0.569	0.522	0.586	0.527	0.458	0.040	0.33	0.57	0.13	0.10
Crude protein	0.854	0.786	0.758	0.802	0.718	0.012	<0.001	0.85	0.33	0.024
Ether extract	0.391	0.619	0.723	0.641	0.669	0.021	<0.001	<0.001	0.44	0.075
aNDFom	0.518	0.496	0.482	0.477	0.418	0.034	0.12	0.83	0.39	0.37
Hemicelluloses[d]	0.538	0.490	0.489	0.497	0.430	0.047	0.18	0.81	0.60	0.51
Cellulose[e]	0.567	0.504	0.512	0.486	0.395	0.035	0.013	0.67	0.076	0.19
Total polyphenols	0.663	0.791	0.855	0.774	0.810	0.144	<0.001	0.31	0.38	0.70
Starch	0.986	0.980	0.973	0.980	0.974	0.0034	0.013	0.94	0.85	0.92
Gross energy	0.877	0.821	0.797	0.825	0.767	0.0087	<0.001	0.44	0.14	0.054
UE/DE[f]	0.028	0.036	0.045	0.041	0.048	0.0032	<0.001	0.68	0.19	0.80

[a]100_COC = 100g/kg crude OC; 200_COC = 200g/kg crude OC; 100_PDOC = 100g/kg partially defatted OC; 200_PDOC = 200g/kg partially defatted OC.
[b]Contrasts: 1 = linear effect of level of OC , 2 = quadratic effect of level of OC , 3 = type of OC, 4 = interaction type * level of OC.
[c]Standard error of means (n = 6).
[d]Calculated as the difference between ADFom and aNDFom.
[e]Calculated as the difference between ADFom and ADL.
[f]Proportion of digestible energy lost in urine.

524 **Table 5** *Effect of type and level of inclusion of olive cake (OC) on faeces and urine composition (g/kg DM)*

	Diets[a]						Significance[b]			
	Basal	100_COC	200_COC	100_PDOC	200_PDOC	SEM[c]	1	2	3	4
Faeces										
Dry matter	400	383	381	402	401	13.5	0.54	0.80	0.15	0.96
Ash	195	175	147	172	160	1.05	0.003	0.97	0.63	0.46
Crude protein	230	233	226	224	224	5.45	0.48	0.83	0.29	0.59
Ether extract	88.5	70.0	63.9	63.6	59.0	3.12	<0.001	0.009	0.078	0.82
aNDFom	474	460	464	485	482	19.1	0.99	0.94	0.29	0.86
ADFom	196	225	258	246	269	11.8	<0.001	0.64	0.21	0.68
ADL	63	82	113	97	114	7.91	<0.001	0.91	0.37	0.41
Hemicelluloses[d]	278	235	207	239	213	16.3	0.002	0.68	0.75	0.93
Cellulose[e]	133	142	144	149	154	6.03	0.039	0.48	0.19	0.81
Polyphenols	0.099	0.176	0.242	0.180	0.274	0.30	<0.001	0.87	0.44	0.61
Starch	50.7	52.3	52.3	50.7	46.0	6.14	0.85	0.79	0.59	0.71
Gross energy, MJ	18.0	19.2	20.2	19.4	20.2	0.084	<0.001	0.055	0.45	0.28
pH	6.56	6.51	6.49	6.42	6.43	0.079	0.28	0.53	0.33	0.80
Urine										
Dry matter	5.58	5.73	6.16	5.25	5.83	0.659	0.58	0.61	0.51	0.93
Total Kjeldahl N, mg/mL	11.3	8.71	7.20	8.34	7.31	0.48	0.008	0.46	0.99	0.82

Benzoic acid, mg/mL	0.408	0.659	0.796	0.432	0.779	0.123	0.005	0.28	0.82	0.46
Hippuric acid, mg/mL	0.647	0.774	1.73	0.918	1.46	0.273	0.013	0.65	0.33	0.40
pH	8.20	8.12	7.68	8.09	7.80	0.184	0.047	0.45	0.80	0.69
Gross energy, MJ/kg DM	8.26	9.36	10.9	10.1	11.1	0.313	<0.001	0.70	0.09	0.35

525 [a]100_COC = 100g/kg crude OC; 200_COC = 200g/kg crude OC; 100_PDOC = 100g/kg partially defatted OC; 200_PDOC = 200g/kg partially defatted OC.
526 [b]Contrasts: 1 = linear effect of level of OC , 2 = quadratic effect of level of OC , 3 = type of OC, 4 = interaction type * level of OC.
527 [c]Standard error of means (n = 6).
528 [d]Calculated as the difference between aNDFom and ADFom.
529 [e]Calculated as the difference between ADFom and ADL.
530
531
532

533 **Table 6** *Effect of type and level of inclusion of olive cake (OC) on DM and nitrogen (N) balance*

	Diets[a]						Significance[b]			
	Basal	100_COC	200_COC	100_PDOC	200_PDOC	SEM[c]	1	2	3	4
Mean body weight (kg)	83.0	80.8	82.0	84.2	77.9	1.49	0.10	0.47	0.80	0.02
DM balance g/kg$^{0.75}$										
Intake	63.1	67.5	66.4	72.9	73.3	4.36	0.21	0.37	0.16	0.86
Faeces	7.91	11.6	12.7	12.2	16.2	1.08	<0.001	0.47	0.07	0.19
Urine	3.51	3.88	4.15	4.58	4.69	0.18	0.09	0.48	0.14	0.84
N balance, g/kg$^{0.75}$										
Intake	1.98	2.03	1.90	2.24	2.04	0.13	0.94	0.18	0.18	0.77
Faeces	0.293	0.430	0.461	0.442	0.581	0.04	<0.001	0.46	0.13	0.20
Urine	0.726	0.568	0.485	0.706	0.566	0.03	0.02	0.85	0.12	0.67
Retained	0.963	1.04	0.957	1.09	0.891	0.04	0.58	0.15	0.83	0.50

534 [a]100_COC = 100g/kg crude OC; 200_COC = 200g/kg crude OC; 100_PDOC = 100g/kg partially defatted OC; 200_PDOC = 200g/kg partially defatted OC.

535 [b]Contrasts: 1 = linear effect of level of OC , 2 = quadratic effect of level of OC, 3 = type of olive cake, 4 = interaction type * level of OC.

536 [c]Standard error of means (n = 6).

537

538 **Table 7** *Effect of type and level of inclusion of olive cake (OC) on slurry (faeces+urine) excretion, initial characteristics and derived ammonia*

539 *(NH_3) emission and Biochemical Methane (CH_4) Potential.*

	Diets[a]						Significance[b]			
	Basal	100_COC	200_COC	100_PDOC	200_PDOC	SEM[c]	1	2	3	4
Slurry excretion (kg/day)	2.23	2.54	2.77	2.98	3.17	0.25	0.01	0.48	0.10	0.92
Slurry characteristics										
Dry matter (g/kg)	111	129	151	116	152	13.0	0.01	0.49	0.63	0.61
Organic matter (g/kg)	81.6	111	121	90.0	122	10.4	0.001	0.90	0.33	0.26
Total ammonia nitrogen (g/L)	9.11	6.32	3.88	5.72	5.19	0.73	<0.001	0.25	0.64	0.21
Total Kjeldahl nitrogen (g/kg)	11.8	9.49	8.58	9.09	8.84	0.88	0.01	0.23	0.93	0.71
pH	8.61	8.26	7.68	8.07	7.90	0.09	<0.001	0.70	0.85	0.04
Total volatile fatty acids (mmol/L)	52.8	56.1	60.9	48.3	54.5	5.72	0.47	0.57	0.23	0.91
Acetic acid (mmol/L)	39.3	42.5	46.0	37.3	41.0	4.14	0.40	0.69	0.22	0.98
Propionic acid (mmol/L)	6.56	6.27	6.80	5.45	5.47	0.86	0.68	0.55	0.22	0.77
Butyric acid (mmol/L)	2.87	3.01	4.26	2.38	3.59	0.68	0.20	0.27	0.35	0.98
Gas emissions										
g NH_3/kg slurry	1.76	1.22	0.97	1.35	1.01	0.10	<0.001	0.34	0.40	0.67
g N-NH_3/kg initial Total Kjeldahl nitrogen	163	138	116	149	119	11	0.002	0.72	0.49	0.71
mg NH_3/animal and day	359	356	269	393	303	33	0.08	0.10	0.29	0.97

Biochemical methane potential										
B_0, mL CH_4/g OM	300	286	266	280	274	13.7	0.05	0.87	0.93	0.58
L CH_4/animal and day	52.6	76.7	92.4	77.2	110	6.11	<0.001	0.98	0.12	0.14

[a]100_COC = 100g/kg crude OC; 200_COC = 200g/kg crude OC; 100_PDOC = 100g/kg partially defatted OC; 200_PDOC = 200g/kg partially defatted OC.

[b]Contrasts: 1 = linear effect of level of OC inclusion, 2 = quadratic effect of level of OC inclusion 3 = type of OC, 4 = interaction type * level of OC.

[c]Standard error of means (n = 6).