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[Ferrer, P., Calvet, S., García-Rebollar, P., de Blas, C., Jiménez-Belenguer, A. I., Hernández, P., ... & Cerisuelo, A. (2020). Partially defatted olive cake in finishing pig diets: Implications on performance, faecal microbiota, carcass quality, slurry composition and gas emission. *Animal*, 14(2), 426-434.]

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The final publication is available at

[\[http://dx.doi.org/10.1017/S1751731119002040\]](http://dx.doi.org/10.1017/S1751731119002040)

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1 Partially defatted olive cake in finishing pig diets: implications on 2 performance, faecal microbiota, carcass quality, slurry composition 3 and gas emission

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12 (Received 3 November 2018; Accepted 16 July 2019)

14

15 *One of the key factors to improve swine production sustainability is the use of agro-industrial by-products in feeds, such as olive AQ4*
16 *by-products. However, it is necessary to assess its effects on the overall production process, including the animal and the*
17 *environment. With this aim, an experiment was conducted to determine the effects of including a partially defatted olive cake*
18 *(PDOC) in pig diets on growth performance, faecal microbiota, carcass quality and gas emission from the slurry. Two finishing*
19 *diets were formulated to be isonutritive, a control (C) diet and a diet with PDOC included at 120 g/kg. Eighty finishing male pigs*
20 *Duroc-Danbred × (Landrace × Large White) of 60.4 ± 7.00 kg BW were divided between these two treatments. During the*
21 *finishing period (60 to 110 kg BW, 55 days) average daily gain, average daily feed intake and feed conversion ratio were*
22 *recorded. Faecal samples from the rectum of 16 animals per treatment were incubated for bacteria enumeration. At the end of*
23 *finishing period, backfat thickness and loin depth (LD) were measured. Animals were slaughtered to obtain carcass weight and*
24 *carcass composition parameters, and subcutaneous fat was sampled to analyse the fatty acid (FA) profile. In addition greenhouse*
25 *gas and ammonia emissions were measured during 2 months of pig slurry storage using the methodology of dynamic flux*
26 *chambers. An initial slurry characterisation and biochemical methane potential (B₀) were also determined. No significant*
27 *differences between treatments were found in performance, carcass quality and microbial counts with the exception of LD, which*
28 *was lower in PDOC compared with C animals (45.5 v. 47.5 mm, SEM: 0.62; P = 0.020). The FA profile of the subcutaneous fat*
29 *did not differ between treatments, but the monounsaturated fatty acid (MUFA) concentration was higher and the*
30 *polyunsaturated FA was lower in the animals fed PDOC (50.9 v. 48.3, SEM: 0.48, P < 0.001; 17.6 v. 19.3, SEM: 0.30, P < 0.001 AQ5*
31 *for PDOC and C animals, respectively). The initial pig slurry characterisation only showed differences in ADF concentration that was*
32 *higher (P < 0.05) in the slurry from PDOC treatment. Regarding gas emission, slurries from both treatments emitted similar amounts*
33 *of ammonia (NH₃), carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), as well as B₀ values. The results obtained suggest*
34 *that PDOC may be included in balanced pig diets at rates of up to 120 g/kg without negative effects on performance, carcass*
35 *quality, gut microflora and slurry gas emission, while improving the MUFA concentration of subcutaneous fat.*

36 **Keywords:** olive by-products, swine, growth performance, carcass traits, gaseous emissions

37 Implications

38 The use of olive cake (OC) in animal feed can be of interest for
39 the livestock sector, increasing its profitability and sustain-
40 ability. Moreover its oleic acid and polyphenols' content
41 might positively affect carcass traits and gut health. From

the results obtained in the present work, partially defatted 42
olive cake can play a role in pig nutrition, since neither per- 43
formance or carcass quality traits nor the environmental 44
impact of slurries was negatively affected by its inclusion 45
in diets. Moreover, its use improves the monounsaturated 46
fatty acid concentration in subcutaneous fat. This knowledge 47
is essential to implement the use of OC in animal feeding and 48

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49 to find more sustainable feeding strategies in the livestock
50 sector.

51 Introduction

52 The use of agro-industrial by-products in animal feed can be
53 economically and environmentally beneficial to the livestock
54 sector, increasing its profitability and sustainability. Olive
55 cake (OC) is one of the most relevant agro-industrial by-
56 products in the Mediterranean area, and a major pollutant
57 from olive oil production. The combination of environmental
58 concerns of OC management and the economic interest of
59 components such as phenols has raised the research activity
60 on this by-product (García-González and Aparicio, 2010).
61 Animal feeding is considered one of its possible end
62 uses, and this would contribute to circular economy in
63 Mediterranean countries such as Spain, the most important
64 olive oil producer and the fourth-biggest pig producer world-
65 wide (FAOSTAT, 2017). Olive cake consists of olive pulp, skin,
66 stone and water. Stones represent about 18% to 32% of the
67 product and are generally removed and used as biomass
68 (FEDNA, 2010). In general, OCs without stones show a
69 high fibre and lignin content (from 160 to 557 g lignin/kg
70 DM) and a low but variable CP content (44 to 115 g/kg
71 DM) (Alburquerque *et al.*, 2004; Molina-Alcaide and
72 Yáñez-Ruiz, 2008; De Blas *et al.*, 2015a). Its oil content
73 depends on the oil extraction procedure. The crude OC con-
74 tains about 120 to 140 g/kg ether extract (EE), and it can be
75 partially or totally extracted, based on variability in the mar-
76 ket price of olive oil (Molina-Alcaide and Yáñez-Ruiz, 2008;
77 De Blas *et al.*, 2015b). The fatty acid (FA) composition of OC
78 reveals a high proportion of oleic acid (Joven *et al.*, 2014) and
79 thus a possible positive effect on the quality of animal prod-
80 ucts when used in diets. In main producing areas of olive oil
81 in Spain, these by-products are generally dried and available
82 throughout the year and, thus, potentially used as a source of
83 energy in finishing pigs and sows. Recent studies in growing-
84 finishing pigs show that its digestible energy (DE) content is
85 variable depending on its oil content (around 60% to 80% of
86 the DE provided by barley grain; Ferrer *et al.*, 2018) and that
87 its inclusion up to 100 g/kg of OC in diets replacing barley
88 grain on a weight basis does not impair feed intake and
89 growth but decreases dietary DE concentration and carcass
90 conformation and backfat thickness (Joven *et al.*, 2014). In
91 addition, its use as a feed ingredient might modify slurry pro-
92 duction, composition and gas emission (Ferrer *et al.*, 2018).
93 These effects might be related to its high fibre and phenolic
94 content. Previous research has shown that including fibrous
95 by-products (e.g. sugar beet pulp, orange pulp, carob meal or
96 rapeseed meal) in diets for growing pigs can help to reduce
97 ammonia (NH₃) and occasionally methane (CH₄) emission
98 from faeces or slurry, per unit of nitrogen (N) or organic mat-
99 ter (OM), respectively (Canh *et al.*, 1997; Torres-Pitarch *et al.*,
100 2014; Beccaccia *et al.*, 2015). In the case of OC its inclusion in
101 diets might also decrease NH₃ emission from slurry (Ferrer
102 *et al.*, 2018). On the other hand, olive by-products are rich
103 in phenolic components (3.0 to 50.0 g/kg DM) with a high

antimicrobial and antioxidant capacity (Leouifoudi *et al.*, 104
2015). When included in diets this antimicrobial capacity 105
might also affect animal health and bacterial-dependent 106
gas emission from slurry. 107

The objective of the present study was to determine the 108
effects of the inclusion of a partially defatted olive cake 109
(PDOC) in balanced finishing pig diets on growth perfor- 110
mance, carcass quality, faecal microbiology, slurry composi- 111
tion and gas emission. 112

Material and methods 113

Animals, diets and experimental design 114

Eighty growing males, progeny of Duroc-Danbred × 115
(Landrace × Large White) at 25.1 ± 3.6 kg initial BW were 116
used in the experiment. At arrival, pigs were identified and 117
distributed according to BW in 16 pens and 2 rooms (8 pens 118
per room). The slurry pit from one of the rooms was divided 119
into four different pits that allowed the collection of the slurry 120
excreted by the animals housed in two consecutive pens and 121
fed with the same diet. All the animals were phase-fed two 122
common commercial feeds before the beginning of the exper- 123
imental period (phase 1: from 25 to 34 kg BW; phase 2: from 124
34 to 61 kg BW). At 60.5 kg BW pens were assigned to two 125
different treatments (eight pens/treatment according to aver- 126
age pen weight and SD within pen). These treatments con- 127
sisted of a control feed (C-diet) or a feed with 120 g/kg of 128
PDOC (PDOC-diet) formulated to be isocaloric and isoami- 129
noacidic by adjusting the added fat, soybean meal and syn- 130
thetic amino acids. Minerals were also adjusted to 131
requirements in both diets. The OC inclusion level in the 132
PDOC diet was chosen from the results obtained in the study 133
of Joven *et al.* (2014) and our previous results (Ferrer *et al.*, 134
2018) reporting no differences in average daily feed intake 135
(ADFI) up to 200 g/kg inclusion level of PDOC. Detailed 136
OC and experimental diets composition are given in 137
Tables 1 to 4. The dehydrated OC was obtained from an olive 138
pomace industry (DCOOP, Antequera, Spain) and added in 139
the PDOC-diet at the expense of barley and sunflower meal. 140
The coefficient of total tract apparent digestibility (CTAD) 141
of energy for OC was previously determined in an *in vivo* 142
study (Ferrer *et al.*, 2018). Experimental feeds were offered 143
ad libitum in dry form (pelleted) for 55 days, until slaughter 144
(118 ± 10.6 kg BW). Free access to water was provided 145
during all of the experimental period. 146

Growth performance, carcass and meat quality 147

Pigs were individually weighed fortnightly from the start of 148
trial until slaughter. Feed consumption was recorded and 149
the average daily gain (ADG), ADFI and feed conversion ratio 150
(FCR) were then calculated. *In vivo* backfat (BF) and loin 151
depth (LD) were measured at the P2 position, using a B-mode 152
ultrasound device (Agroscan A16, Angoulême, France) as 153
described by Cerisuelo *et al.* (2010) on days 53 to 54. At 154
the end of the experimental period pigs were slaughtered. 155
Fasting was practised for approximately 12 h before 156

Table 1 Chemical composition of the partially defatted olive cake used in the swine trial (g/kg DM, unless otherwise specified)

Analysed chemical composition	OC
Dry matter (g/kg FM)	914
Ash	121
Gross energy, MJ/kg	23.0
Digestible energy, MJ/kg	8.45
Crude protein	92.3
Digestible crude protein	21.0
Ether extract	122
NDF ¹	415
ADF ¹	290
Lignin	171
NDICP ²	61.7
ADICP ³	37.6
Total polyphenols ⁴	8.6
Sugars	83.2

OC = olive cake; NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP.

¹Ash-free.

²Neutral detergent insoluble CP.

³Acid detergent insoluble CP.

⁴Expressed as acid gallic.

slaughter in all animals. Carcass weight (hot carcass weight) and carcass composition were measured using an ultrasonic automatic carcass grading device (AutoFom™, Carometec food technology, Denmark) following the methodology described by Torres-Pitarch *et al.* (2014). At approximately 2 h *postmortem* (during the chilling process) pH in the *splenius* muscle and meat colour components at the *gracilis* muscle were recorded using a pH meter (model ph25+, Crison, Barcelona, Spain) and a portable CR300 Minolta Chromameter (Konica Minolta, Osaka, Japan), respectively. In addition, subcutaneous fat was sampled at the level of the second cervical vertebrae to analyse the FA profile at the left side of the carcass of 20 animals per treatment as described by Torres-Pitarch *et al.* (2014).

Faecal microbiota by culture-based methods

Faecal samples were aseptically removed directly from the rectum of 16 animals per treatment (2 animals per pen) at days 35 and 36 of the experimental period for bacterial (total anaerobic bacteria, *Enterobacteria*, *Lactobacilli* and *Bifidobacteria*) enumeration. The samples of each pen were pooled and treated as a pen sample. Within the 2 h after collection, faecal samples were diluted 1 : 10 (1 g faeces in 9 ml of peptone water) and decimal dilutions were prepared. The number of colony forming units per gram (CFU/g faeces) of total anaerobic bacteria and *Bifidobacteria* was isolated onto Thioglycolate Agar (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) and BD *Bifidobacterium* Agar, Modified (Becton Dickinson GmbH, Germany), respectively, following anaerobic incubation at 37°C for 72 h. *Enterobacteria* were isolated on McConkey agar (Liofilchem), following aerobic incubation at 37°C for 24 h. *Lactobacilli* were cultured on Man, Rogosa, Sharp agar (MRS, Liofilchem) following incubation at 37°C for 48 h. All colonies were counted immediately after removal from the incubator.

Table 2 Fatty acid profile of the of the partially defatted olive cake used in the swine trial (g/kg DM)

Analysed chemical composition	OC
Total fatty acids	121
Saturated fatty acids	19.0
Lauric acid (C12:0)	0.51
Myristic acid (C14:0)	0.29
Palmitic acid (C16:0)	12.9
Heptadecanoic acid (C17:0)	1.22
Stearic acid (C18:0)	4.11
Monounsaturated fatty acids	88.0
Palmitoleic acid (C16:1n7)	0.92
Heptadecenoic acid (C17:1)	1.36
Oleic acid (C18:1n9)	83.8
Vaccenic (C18:1n7)	1.64
Eicosenoic acid (C20:1n9)	0.20
Docosadienoic acid (C22:1n9)	0.07
Polyunsaturated fatty acids	13.6
Linoleic acid (C18:2n6)	10.5
Linolenic acid (C18:3n3)	1.20
Estearidonic acid (C18:4n3)	0.24
Eicosatrienoic acid (C20:3n9)	0.04
Arachidonic acid (C20:4n6)	0.08
Eicosapentaenoic acid (C20:5n3)	0.10
Docosatetraenoic acid (C22:4n6)	0.12
Docosapentaenoic acid (C22:5n3)	0.87
Docosahexaenoic acid (C22:6n3)	0.46

OC = olive cake.

Slurry measurements and gas emission

At the end of the fattening period, the slurry excreted from each individualised pit (two pits per treatment) was quantified by measuring the level of the slurry achieved in the pit. Afterwards, the slurry in each pit was homogenised with a pump, and a representative sample was pumped to two tanks of 120 l of capacity per pit (470 mm diameter and 800 mm height) leaving a 200 mm of headspace between the slurry surface and the top of the tank. Overall, eight tanks were filled with 90 l of slurry each and sampled during the fill-in for slurry chemical characterisation. The tanks were placed in a mechanically ventilated room for 8 successive weeks simulating outdoor slurry storage. The gas emissions (NH₃, CH₄, carbon dioxide (CO₂) and nitrous oxide (N₂O)) from slurry over the storage period were measured using the methodology described by Calvet *et al.* (2017). In brief, tanks were set as a dynamic chamber by fitting specially adapted lids which had a central circular hole connected to a fan with an extraction duct to draw air from the tank headspace. The lids were only placed on the tanks over the gas measurement periods, remaining open the rest of the time to simulate natural storage conditions. Gas concentrations were measured at the outlet duct of each tank and at the room ambient by means of a photoacoustic gas monitor (INNOVA1412, Air Tech Instruments, Ballerup, Denmark) connected to a multi-point sampler. Every week, emissions were measured continuously during 48 h for the eight tanks.

Table 3 Ingredient content and chemical composition of the experimental pig diets (g/kg as fed, unless otherwise specified)

Ingredients	Treatments ¹	
	C-diet	PDOC-diet
Barley	455	332
Triticale	50.0	50.0
Wheat	150	150
Hominy feed	86.0	86.0
Glycerol	10.0	10.0
Rapeseed meal	86.0	86.0
Sunflower meal	60.0	20.0
Soybean meal	30.0	60.0
Partially defatted olive cake	0	120
Fat	43.0	58.0
Calcium carbonate	11.5	8.3
Sodium chloride	3.6	3.3
Monocalcium phosphate	0	1.2
L-lysine	6.4	6.0
Methionine	0.7	1.1
Threonine	1.4	1.5
Tryptophan	0.1	0.2
Valine	0	0.1
Phytase	0.2	0.2
Liquid acid	0.15	0.15
Vitamin-mineral premix ²	0.50	0.50
Analysed chemical composition, g/kg DM		
Dry matter (g/kg FM)	906	908
Ash	43.6	51.9
Crude protein	171	161
Ether extract	85.9	119
NDF ³	182	215
ADF ³	59.2	74.3
Lignin	20.4	32.5
NDICP ⁴	24.5	33.2
ADICP ⁵	1.6	4.4
Total polyphenols	0.38	0.98
Gross energy, MJ/kg	17.7	18.4
Calculated chemical composition ⁶		
Digestible energy, kcal/kg ⁷	3341	3277
Net energy, kcal/kg ⁷	2411	2412
Calcium	0.58	0.59
Phosphorus	0.42	0.41
A Q6 Ileal standardised ileal amino acids		
Lysine	0.78	0.78
Methionine	0.26	0.28
Methionine + Cystine	0.48	0.48
Threonine	0.51	0.51
Tryptophan	0.14	0.14
Valine	0.51	0.51

PDOC = partially defatted olive cake; NDICP = neutral detergent insoluble CP; ADICP = acid detergent insoluble CP.

¹C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.

²Vitamin-mineral premix in the finishing phase provided per kilogram of feed: retinol, 6500 IU (E672); cholecalciferol, 1860 IU (E671); α -tocopherol, 10 mg; menadione, 0.6 mg; thiamine, 0.8 mg; riboflavin, 3.2 mg; pyridoxin, 1.0 mg; cobalamin, 0.02 mg; niacin, 12 mg; pantothenic acid, 9.60 mg; choline chloride, 116 mg; Fe, 72 mg as FeSO₄·7H₂O; Cu, 16 mg as CuSO₄·5H₂O; Zn, 80 mg as ZnO; Mn, 40 mg as MnO; I, 1.44 mg as KI and Se, 0.20 mg as Na₂SeO₃.

³Ash-free.

⁴Neutral detergent insoluble CP.

⁵Acid detergent insoluble CP.

⁶Calculated values based on De Blas *et al.* (2015b).

⁷Calculated from the coefficient of total tract apparent digestibility of energy previously determined in Ferrer *et al.* (2018).

Table 4 Fatty acid profile of the experimental pig diets (g/kg DM)

	Treatments ¹	
	C-diet	PDOC-diet
Total fatty acid	64.4	89.6
Saturated fatty acids	20.9	31.6
Capric acid (C10:0)	0.02	0.03
Lauric acid (C12:0)	0.03	0.06
Myristic acid (C14:0)	0.65	1.29
Pentadecanoic acid (C15:0)	0.06	0.17
Palmitic acid (C16:0)	13.8	18.9
Heptadecanoic acid (C17:0)	0.22	0.54
Stearic acid (C18:0)	5.74	10.2
Arachidic acid (C20:0)	0.17	0.21
Behenic acid (C22:0)	0.10	0.11
Lignoceric acid (C24:0)	0.05	0.07
Monounsaturated fatty acids	23.3	38.4
Palmitoleic acid (C16:1)	1.01	1.33
Myristoleic acid (C14:1)	0.03	0.09
Heptadecenoic acid (C17:1)	0.11	0.24
Oleic acid (C18:1n9c)	19.1	31.6
Vaccenic acid (C18:1n7)	2.21	3.09
Elaidic acid (C18:1n9t)	0.47	1.57
Eicosenoic acid (C20:1)	0.38	0.42
Erucic acid (C22:1n9)	0.03	0.08
Polyunsaturated fatty acids	20.1	19.5
Linoleic acid (C18:2n6c)	18.6	17.8
Linolenic acid (C18:3n3)	1.16	1.41
Eicosadienoic acid (C20:2)	0.19	0.14
Eicosatrienoic acid (C20:3n6)	0.03	0.04
Arachidonic acid (C20:4n6)	0.08	0.08
Docosatetraenoic acid (22:4n6)	0.03	0.03
Docosapentaenoic acid (22:5n3)	0.02	0.03
Docosahexaenoic acid (C22:6n3)	0	0.03
PUFA/SFA	0.96	0.62
MUFA/SFA	1.11	1.22
Oleic acid/total fatty acids	0.297	0.353

PDOC = partially defatted olive cake; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids.

¹C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.

Ammonia concentration measurements were also verified 217 using acid wet traps. A subsample from the exhausted air 218 from the headspace of the tanks was forced to pass with 219 an air pump through absorption flasks filled with 100 ml 220 of 0.05N H₂SO₄. The quantity of total ammonia N (TAN) 221 trapped in the absorption flasks was analysed following 222 4500 NH₃-D procedure (APHA, 2005) using a detection elec- 223 trode (Orion High Performance NH₃ Electrode, model 224 9512HPBNWP, Thermo Scientific, USA). 225

In addition, the biochemical CH₄ potential (B₀) from the 226 initial pit slurry sampled during the fill in of the tanks was 227 measured. 228

Chemical analysis

The PDOC and experimental feeds were analysed for DM, ash 230 and EE according to the Association of Official Analytical 231 Chemists (AOAC, 2000) procedures. Total sugars were 232

233 analysed according to the method of Yemm and Willis (1954).
 234 The concentrations of NDF, ADF and ADL were determined
 235 sequentially according to Van Soest procedure (Van Soest
 236 *et al.*, 1991). The gross energy (GE) concentration was mea-
 237 sured in an isoperibol bomb calorimeter (Parr 6400, Parr
 238 Instruments Co., Moline, IL, USA). Total N was measured by
 239 combustion using Leco equipment (model FP-528, Leco
 240 Corporation, St. Joseph, MI, USA) and CP estimated as
 241 N content \times 6.25. The proportion of neutral and acid detergent
 242 insoluble CP (NDICP and ADICP, respectively) was determined
 243 following the standardised procedures in Licitra *et al.* (1996).
 244 Feeds and PDOC samples were defatted with petroleum ether
 245 prior to fibre analysis. The polyphenolic compounds were
 246 determined after extraction with methanol/acetone/water fol-
 247 lowing the procedure described by Chamorro *et al.* (2012) and
 248 results expressed as gallic acid equivalent.

249 Slurry samples were analysed for pH in duplicate using a
 250 glass electrode (Crison Basic 20+, Crison) and for DM, ash
 251 and OM, EE, fibre and GE following the same methodology used
 252 for PDOC and feeds. The TAN and total Kjeldahl N (TKN) were
 253 analysed by steam distillation (4500 NH3-B and 4500 NH3-C
 254 procedures; APHA, 2005) using an automatic analyser (2300
 255 Kjeltec, Foss Analytical, Hilleroed, Denmark). To avoid N volati-
 256 lisation, the subsample used for TAN analyses was acidified with
 257 HCl immediately after the samples were collected.

258 The B_0 from the slurry was measured in a batch assay,
 259 using 120 ml glass bottles incubated at a mesophilic range
 260 ($35^\circ\text{C} \pm 1^\circ\text{C}$) for 100 days, following the methodology
 261 described by Ferrer *et al.* (2018). Anaerobic inoculum was
 262 collected from an anaerobic digester that treats domestic
 263 and industrial wastewater from the wastewater treatment
 264 plant in Sagunto (Spain), and pre-incubated for 15 days at
 265 35°C in order to deplete the residual biodegradable organic
 266 material (degasification). An inoculum to substrate ratio of
 267 1 on OM basis was used.

268 The FA profile of the PDOC, experimental feed samples and
 269 the subcutaneous fat was measured by gas chromatography.
 270 Fatty acid methyl esters (FAME) were prepared according to
 271 O'Fallon *et al.* (2007) and were analysed in a Focus Gas
 272 Chromatograph (Thermo, Milan, Italy). The FA profile was cal-
 273 culated as the proportion of saturated, monounsaturated and
 274 polyunsaturated FA (SFA, MUFA and PUFA, respectively) in
 275 grams per 100 g of FA.

276 *Statistical analysis*

277 Data were analysed using SAS[®] (Statistical Analysis System)
 278 System Software (Version 9.1, SAS Institute Inc., Cary, NC,
 279 EEUU). Differences in BW, ADG, ADFI, FCR, BF, LD and car-
 280 cass and meat quality traits between experimental treat-
 281 ments were tested by one-way ANOVA using the GLM
 282 procedure of SAS in a completely randomised block design,
 283 with the dietary treatment (C-diet and PDOC-diet) as the
 284 main effect and room as a block factor in the models.
 285 Microbial counts were \log_{10} transformed before analysis.
 286 For ADG, ADFI, FCR, initial and final weight, and microbial
 287 counts, the experimental unit was the pen, and for the car-
 288 cass and meat quality measurements the individual pig was

considered the experimental unit. Gas emission results (mg/l 289
 and h) are presented as the average emission rate during the 290
 experiment. These data were also calculated in mg/animal 291
 day and h (considering the total amount of slurry excreted), 292
 and both were statistically analysed, together with initial 293
 slurry characterisation, by a one-way ANOVA using the 294
 GLM procedure of SAS, where the pit was the experimental 295
 unit and the dietary treatment was considered the source of 296
 variation. 297

298 **Results**

The statistical analysis performed showed no significant 299
 influence ($P > 0.10$) of the room and its interaction with 300
 the dietary treatment for any of the traits studied (data 301
 not shown), so that this effect was excluded from the model. 302

303 *Partially defatted olive cake and experimental diets*

304 *composition*
 The chemical composition of the OC used in the study is sum- 305
 marised in Tables 1 and 2. This OC presented a high EE 306
 (122 g/kg DM), sugar (82.2 g/kg DM) and DE (8.45 MJ/kg 307
 DM) content. Its fibre concentration was also high, particu- 308
 larly its ADL level. The analysed FA profile of OC revealed that 309
 oleic acid was the main FA (83.8 g/kg), followed by palmitic 310
 and linoleic acids. Regarding the chemical composition of the 311
 experimental diets (Tables 3 and 4), PDOC-diet showed a 312
 39% higher EE, fibre (18% and 26% higher content of the 313
 NDF and ADF fractions), and lignin and polyphenol content 314
 compared with C-diet. The concentration of almost all FA 315
 (especially oleic acid) was also greater in the PDOC-diet 316
 compared with C-diet. 317

318 *Growth performance, carcass and meat quality*

319 The results on growth performance are summarised in
 320 Table 5. At the end of the study, BW was not significantly
 321 different between treatments. No significant differences were
 322 obtained in ADG or in ADFI. However, FCR tended to be
 323 higher (0.12 units; $P = 0.059$), and LD was significantly lower
 324 (2.02 units, $P = 0.02$) in the group of animals offered PDOC-
 325 diet. The carcass and meat quality traits measured are shown
 326 in Table 6. The inclusion of PDOC in pig diets had no signifi-
 327 cant effect on carcass characteristics, except the pH that was
 328 lower ($P < 0.001$) and the red colour (a^*) that tended
 329 ($P = 0.086$) to be lower in the meat of pigs offered PDOC-
 330 diet. Regarding the FA profile of the subcutaneous fat
 331 (Table 7), total MUFA concentration was higher and total
 332 PUFA concentration lower in the fat tissue of animals offered
 333 the diet with 12% PDOC compared with that of the C-diet
 334 ($P < 0.001$). The ratio MUFA/SFA was higher and the ratio
 335 PUFA/SFA lower in the pigs offered PDOC compared with
 336 the pigs offered C-diets ($P < 0.05$). Taking into account indi-
 337 vidual FA, the biggest differences between treatments were
 338 found for the MUFA acids, especially palmitoleic, heptadeca-
 339 noic, oleic and vaccenic acids ($P < 0.05$), in the fat of animals
 340 offered PDOC compared with that of the animals offered

Table 5 Effect of the inclusion of partially defatted olive cake in diets on pig performance traits

	Treatments ¹		SEM	P-value
	C-diet	PDOC-diet		
Initial body weight, kg	60.0	60.4	2.24	0.892
Final body weight, kg	119	117	2.27	0.656
Average daily gain, kg/d	1.06	1.03	0.02	0.221
Average daily feed intake, kg/d	2.88	2.93	0.05	0.509
Feed conversion ratio	2.73	2.85	0.04	0.059
Backfat thickness, mm	12.5	12.1	0.351	0.400
Loin depth, mm	47.5	45.5	0.617	0.020

PDOC = partially defatted olive cake.

¹C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.

Table 6 Effect of partially defatted olive cake inclusion in pig diets on carcass and meat quality

	Treatments ¹		SEM	P-value
	C-diet	PDOC-diet		
Carcass characteristics				
Carcass weight, kg	85.8	84.7	1.39	0.580
Carcass yield, %	72.2	72.3	0.268	0.752
Fat depth at GM, mm	1.59	1.59	0.077	0.952
Lean meat percentage, %	58.7	58.3	0.445	0.521
Ham lean meat, mm	71.6	71.6	0.371	0.954
Ham fat, mm	11.5	11.6	0.377	0.970
Bacon lean meat, mm	56.0	55.7	0.578	0.736
Loin lean meat, mm	59.2	58.4	0.621	0.325
Shoulder lean meat, mm	65.8	65.3	0.360	0.390
Lean meat in the 3 to 4 rib, mm	52.7	51.5	0.628	0.157
Fat in the 3 to 4 rib, mm	16.8	17.2	0.413	0.424
Meat quality				
pH ²	6.55	6.01	0.079	<0.001
Meat colour³				
Lightness (L*)	37.4	37.4	0.400	0.979
Redness (a*)	8.76	8.10	0.277	0.086
Yellowness (b*)	3.00	2.74	0.203	0.347

PDOC = partially defatted olive cake; GM = *Gluteus medius* muscle.

¹C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.

²Measured at the *splenius* muscle level.

³Measured at the *gracillis* muscle level.

341 C-diet. Also, the oleic v. total FA ratio was higher ($P < 0.05$)
342 in the fat from animals offered PDOC than C-diet.

343 **Microbial counts**

344 Bacterial counts from faeces did not show any significant
345 differences ($P > 0.05$) between treatments. The ratio
346 *Lactobacilli* : *Enterobacteria* was also similar in both treat-
347 ments (Table 8).

348 **Slurry composition and gas emission**

349 The amount of slurry produced by the animals offered PDOC
350 tended to be 23% higher than that produced by the animals

Table 7 Effect of partially defatted olive cake inclusion in pig diets on FA content and FA profile in subcutaneous fat of pigs (mg/100 mg fresh tissue, unless otherwise specified)

	Treatments ¹		SEM	P-value
	C-diet	PDOC-diet		
Total FA	65.8	68.8		
SFA, mg/100 g total FA	32.4	31.5	0.42	0.116
Capric acid (C10:0)	0.308	0.284	0.0185	0.360
Lauric acid (C12:0)	0.248	0.205	0.0188	0.100
Myristic acid (C14:0)	7.98	8.80	0.190	0.003
Pentadecanoic acid (C15:0)	0.830	0.819	0.0729	0.913
Palmitic acid (C16:0)	137	140	3.69	0.600
Heptadecanoic acid (C17:0)	3.90	4.08	0.180	0.472
Stearic acid (C18:0)	63.1	63.2	3.11	0.983
MUFA, mg/100 g total FA	48.3	50.9	0.48	<0.001
Palmitoleic acid (C16:1)	2.62	2.95	0.084	0.006
C16:1 (n-7)	16.0	16.8	0.53	0.321
Heptadecenoic acid (C17:1)	3.23	3.56	0.092	0.015
Oleic acid (C18:1n9c)	273	301	6.5	0.004
Vaccenic acid (C18:1n11)	16.0	19.1	0.81	0.011
Eicosenoic acid (C20:1)	6.52	6.92	0.197	0.146
PUFA, mg/100 g total FA	19.3	17.6	0.30	<0.001
Linoleic acid (C18:2n6c)	103	97.5	2.76	0.129
Linolenic acid (C18:3n3)	6.46	6.93	0.183	0.067
Eicosadienoic acid (C20:2)	5.37	5.03	0.145	0.096
Eicosatrienoic acid (C20:3n6)	1.08	1.04	0.030	0.428
Arachidonic acid (C20:4n6)	2.42	2.39	0.077	0.765
Docosadienoic acid (C22:2)	5.43	5.75	0.281	0.416
Docosatetraenoic acid (C22:4n6)	1.35	1.10	0.116	0.125
Docosapentaenoic acid (C22:5n3)	0.928	0.814	0.1247	0.511
Docosahexaenoic acid (C22:6n3)	0.218	0.348	0.0567	0.104
PUFA/SFA	0.596	0.559	0.0125	0.037
MUFA/SFA	1.50	1.62	0.034	0.009
Oleic acid/total FA	4.15	4.37	0.039	<0.001

FA = fatty acid; PDOC = partially defatted olive cake; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

¹C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.

fed the C-diet ($P = 0.088$) (Table 9). Regarding slurry compo- 351
sition, the ADF concentration in the slurry of animals offered 352
PDOC was significantly higher than that of the animals 353
offered C-diet ($P < 0.05$). In addition, ADL concentration in 354
the slurry tended to be higher ($P = 0.06$), and the EE concen- 355
tration in the slurry tended to be lower ($P = 0.08$) in the 356
group of animals offered PDOC. Concerning the gas emis- 357
sions, no significant differences were obtained either on 358
the B₀ values or on the amount of gas emitted expressed 359
per litre of slurry or per animal and day. 360

Table 8 Effect of partially defatted olive cake inclusion in diets on faecal bacteria counts (Log_{10} CFU/g fresh faeces) in finishing pigs

	Treatments ¹		SEM	P-value
	C-diet	PDOC-diet		
Total anaerobic bacteria	8.37	8.11	0.179	0.316
<i>Bifidobacteria</i>	8.75	8.50	0.116	0.154
<i>Enterobacteria</i>	6.92	6.62	0.175	0.237
<i>Lactobacilli</i>	9.11	8.73	0.211	0.228
Ratio <i>Lactobacilli</i> : <i>Enterobacteria</i>	1.32	1.33	0.033	0.847

PDOC = partially defatted olive cake.

¹C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.**Table 9** Effect of partially defatted olive cake inclusion in pig diets on slurry characteristics and gas emission¹

	Treatments ²		SEM	P-value
	C-diet	PDOC-diet		
Slurry production, l/animal and day	4.18	5.14	0.216	0.088
Slurry characteristics				
DM, g/kg	83.9	93.9	3.70	0.196
OM, g/kg	67.3	77.1	3.63	0.197
Total ammonia nitrogen, g/l	5.22	3.88	0.430	0.158
Total Kjeldahl nitrogen, g/kg	6.88	5.72	0.302	0.113
NDF, g/kg of DM	413	422	2.90	0.153
ADF, g/kg of DM	209	233	2.53	0.022
ADL, g/kg of DM	76.6	97.7	3.83	0.060
Ether extract, g/kg of DM	159	144	3.29	0.080
pH	6.70	6.61	0.099	0.568
B ₀ , ml CH ₄ /g of OM	394	333	25	0.226
Concentration of gases emitted, mg/l and h				
Ammonia	0.586	0.634	0.053	0.587
Carbon dioxide	4.59	5.29	0.764	0.583
Methane	0.273	0.191	0.063	0.456
Total gas emission, mg/animal and day				
Ammonia	59.3	78.3	8.46	0.253
Carbon dioxide	454.1	653.1	67.5	0.172
Methane	26.8	23.6	4.99	0.695

PDOC = partially defatted olive cake; OM = organic matter.

¹n = 2.²C-diet = control diet; PDOC-diet = diet with 120 g/kg partially defatted olive cake.

361 Discussion

362 The OC used in the present study shows an intermediate EE
363 level and a high sugar content compared with other OC in the
364 literature (EE ranging from 70 to 170 and sugar content rang-
365 ing from 10 to 19 g/kg, De Blas *et al.*, 2015b). In particular, it
366 is known that the sugar and fat content can be variable
367 among dried OC sources (Abo Omar *et al.*, 2012). While
368 the variability observed in terms of fat content among OC

sources is mainly related to the olive oil extraction system, 369
the variability in sugar content of OC is attributed to the time 370
of OC storage before being dried due to microbial fermenta- 371
tion that takes place during its storage (De Blas *et al.*, 2015b). 372
In terms of fibre, as expected, OC is a fibrous product with a 373
relatively high lignin content. Compared with other fibrous 374
feedstuffs such as rapeseed meal, alfalfa or sunflower meal 375
its fibre and particularly ADL content, in this study, was high 376
(INRA, 2004; FEDNA, 2010). All this led to higher EE and fibre 377
fractions content in the PDOC-diet compared with the control 378
diet. The PDOC-diet showed also a higher oleic acid and poly- 379
phenol content, compared with the control-diet (+1.42 and 380
+0.6 g/kg, respectively) due to the high amount of oleic acid 381
(83.8 g/kg DM) and polyphenols (8.6 g/kg DM) in the PDOC. 382

The inclusion of fibrous by-products in pig diets, such as 383
PDOC, has been related to poorer performance traits espe- 384
cially during the growing phase (Jarrett and Ashworth, 385
2018). However, in the current study, diets were formulated 386
to be isonutritive and, accordingly, the inclusion of a 12% 387
PDOC did not lead to significant differences in growth perfor- 388
mance, although a trend to a slight decrease of feed effi- 389
ciency (FCR 0.12 units higher; $P = 0.06$) was detected in 390
pigs offered PDOC. This might indicate that the net energy 391
value for PDOC was overestimated in the current study, since 392
pigs offered PDOC-diet increased feed consumption to meet 393
their energy requirements. On the other hand, *in vivo* BF mea- 394
surement was similar between treatments, but LD was about 395
2 mm lower ($P < 0.05$) on average in the group of pigs fed 396
PDOC-diet. Joven *et al.* (2014) also showed a linear decrease 397
of fat depots in the carcass as the level of OC increased from 398
0% to 15% in finishing pigs. Mas *et al.* (2010) and González 399
et al. (2012) described no differences in carcass characteris- 400
tics from pigs offered diets enriched with oleic acid and olive 401
pomace oil (65 g/kg), respectively. Differences in carcass fat 402
content are expected when pigs consume more energy than 403
required with high-energy diets or when the indispensable 404
amino acid or protein levels in diets are lower than required 405
(Cámara *et al.* 2016). With respect to meat quality traits, 406
Serra *et al.* (2018) showed similar pH levels and lower yellow- 407
ness (b^*) with the inclusion of olive pomace in pig diets. 408
Despite this our results are in accordance with those authors, 409
only the pH values were affected by feeding OC. Pigs fed OC 410
showed a lower meat pH that could be associated with higher 411
muscle glycogen stores. Although a statistical difference has 412
been observed in the muscle pH at 2 h *postmortem*, it has no 413
practical implications on quality traits (no differences were 414
found in colour parameters). Besides, pH values in the range 415
of 6.6 to 6.0 after 2 h *postmortem* are not associated with the 416
development of pale, soft and exudative (PSE) or dark, firm 417
and dry (DFD) meats (Rosenvold and Andersen, 2003). 418

As expected, PDOC addition led to differences in FA profile 419
of subcutaneous fat, with a higher proportion of MUFA and 420
lower proportion of PUFA with respect to total FA. These 421
differences were caused by the oleic acid increment in the 422
diet, since the deposition of FA in pigs is known to be pri- 423
marily influenced by the FA composition of the diet 424
(Cava *et al.* 1997). The modification of FA profile with the 425

426 addition of olive by-products has been described by numer-
427 ous authors (González *et al.*, 2012; Joven *et al.*, 2014; Serra
428 *et al.*, 2018), being of interest due to the improved sensory
429 quality of meat.

430 Polyphenols are able to modulate the intestinal ecology,
431 influencing host health through the bioactive compounds
432 generated by the colonic microbiota (Marín *et al.*, 2015).
433 *In vitro* animal and human studies conducted with a selection
434 of polyphenols at a determinate concentration reported mod-
435 ifications in the gut microbiome by the inhibition of patho-
436 genic bacteria and the stimulation of the growth of
437 beneficial bacteria due to modifications of gut ecosystem
438 (Cardona *et al.*, 2013). On the other hand, the inclusion of
439 insoluble fibre in diets can have a prebiotic effect in the
440 gut of pigs and modify gut microbiota (Pieper *et al.*,
441 2015). In the present study *Lactobacillus*, *Bifidobacterium*
442 and *Enterobacteria* values were similar to those obtained
443 by Zhao *et al.* (2013). However, no significant effects were
444 found when including PDOC in feeds (a fibre-rich ingredient)
445 on gut microbiology. This could indicate an acclimation of
446 the bacteria to the inclusion of PDOC in the diet or a limita-
447 tion of cultured-based technique to assess the diversity
448 and dynamics of the gastrointestinal microbiota.

449 In terms of slurry production and composition, the inclu-
450 sion of PDOC tended to increase the volume of slurry excreted
451 by the animals due to its high fibre (ADF) content as it has
452 been reported by Morazán *et al.* (2015) in a study conducted
453 to evaluate the effects of reducing dietary CP and increasing
454 NDF. These changes in slurry excretion are probably induced
455 by the increased intake of lignified dietary fibre with PDOC-
456 diet since the slurry from the animals offered PDOC-diet
457 showed higher ADL and a numerically higher DM content.
458 The higher fibre content from PDOC-diet probably resulted
459 in an increase in faecal DM and bulk by virtue of its physical
460 presence and water-holding capacity (Bach Knudsen and
461 Hansen, 1991). However, neither the amount of fibre nor
462 the especially high polyphenol content in diets leads to
463 changes in gas emission from slurry. Although numerically
464 lower B_0 values were observed with the inclusion of PDOC
465 that possibly can be related to the high ADF proportion in
466 the slurry, no statistical difference was obtained as was in
467 CH_4 emission during storage. Accordingly, slurry composition
468 was also similar between treatments. This result increases
469 the interest of PDOC in pig nutrition, since neither growth
470 performance or carcass quality traits nor the environmental
471 impact of slurries was negatively affected by its inclusion in
472 balanced diets. Fibrous by-products such as OC, which is a
473 low-cost by-product from the olive oil industry, represents
474 an opportunity to value wastes from food industries to create
475 further value and thus contribute to the economic, social and
476 environmental sustainability of the animal feeding sector.
477 This study demonstrates that using a relevant proportion
478 of OC in balanced feeds for growing pigs (12%) does not
479 have significant negative effects on performance traits
480 favouring the circular economy strategy, with potential pos-
481 itive effects on pig gut health and meat quality.

Acknowledgements

This project was funded by the Spanish Ministry of Science
and Innovation (AGL2014-56653). Preliminary results from
this work have been published in an abstract form (Ferrer
et al., 2017). Acknowledgements are also expressed to
DCOOP for providing PDOC.

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Declaration of interest

The authors confirm that there are no known conflicts of interest
associated with this publication and there has been no signifi-
cant financial support for this work that could have influenced
its outcome.

Ethics statement

The experimental procedure was approved by the Ethics
Committee of the Universitat Politècnica de València (registra-
tion number 2016/VSC/PEA/00024).

Software and data repository resources

None of the data were deposited in an official repository.

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Science 91, 5280–5286. 626