


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

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One of the key factors to improve swine production sustainability is the use of agro-industrial by-products in feeds, such as olive by-products. However, it is necessary to assess its effects on the overall production process, including the animal and the environment. With this aim, an experiment was conducted to determine the effects of including a partially defatted olive cake (PDOC) in pig diets on growth performance, faecal microbiota, carcass quality and gas emission from the slurry. Two finishing diets were formulated, a control (C) diet and a diet with PDOC included at 120 g/kg. Eighty finishing male pigs Duroc-Danbred × (Landrace × Large White) of 60.4 ± 7.00 kg BW were divided between these two treatments. During the finishing period (60 to 110 kg BW, 55 days) average daily gain, average daily feed intake and feed conversion ratio were recorded. Faecal samples from the rectum of 16 animals per treatment were incubated for bacteria enumeration. At the end of finishing period, backfat thickness and loin depth (LD) were measured. Animals were slaughtered to obtain carcass weight and carcass composition parameters, and subcutaneous fat was sampled to analyse the fatty acid (FA) profile. In addition greenhouse gas and ammonia emissions were measured during pig slurry storage using the methodology of dynamic flux chambers. An initial slurry characterisation and biochemical methane potential (B₀) were also determined. No significant differences between treatments were found in performance, carcass quality and microbial counts with the exception of LD, which 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 did not differ between treatments, but the monounsaturated FA (MUFA) concentration was higher and the 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 in mg/100 g of Total FA, for PDOC and C animals, respectively). The initial pig slurry characterisation only showed differences in ADF concentration that was higher (P < 0.05) in the slurry from PDOC treatment. Regarding gas emission, slurries from both treatments emitted similar amounts of ammonia (NH₃), carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), as well as B₀ values. The results obtained suggest that PDOC may be included in balanced pig diets at rates of up to 120 g/kg without negative effects on performance, carcass quality, gut microflora and slurry gas emission, while improving the MUFA concentration of subcutaneous fat.

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

Implications

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

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

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to find more sustainable feeding strategies in the livestock sector.

Introduction

The use of agro-industrial by-products in animal feed can be economically and environmentally beneficial to the livestock sector, increasing its profitability and sustainability. Olive cake (OC) is one of the most relevant agro-industrial by-products in the Mediterranean area, and a major pollutant from olive oil production. The combination of environmental concerns of OC management and the economic interest of components such as phenols has raised the research activity on this by-product (García-González and Aparicio, 2010). Animal feeding is considered one of its possible end uses, and this 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). Olive cake consists of olive pulp, skin, stone and water. Stones represent about 18% to 32% of the product and are generally removed and used as biomass (FEDNA, 2010). In general, OCs without stones show a high fibre and lignin content (from 160 to 557 g lignin/kg DM) and a low but variable CP content (44 to 115 g/kg DM) (Alburquerque *et al.*, 2004; Molina-Alcaide and Yáñez-Ruiz, 2008; De Blas *et al.*, 2015a). Its oil content depends on the oil extraction procedure. The crude OC contains about 120 to 140 g/kg ether extract (EE), and it can be partially or totally extracted, based on variability in the market price of olive oil (Molina-Alcaide and Yáñez-Ruiz, 2008; De Blas *et al.*, 2015b). The fatty acid (FA) composition of OC reveals a high proportion of oleic acid (Joven *et al.*, 2014) and thus a possible positive effect on the quality of animal products when used in diets. In main producing areas of olive oil in Spain, these by-products are generally dried and available throughout the year and, thus, potentially used as a source of energy in finishing pigs and sows. Recent studies in growing-finishing pigs show that its digestible energy (DE) content is variable depending on its oil content (around 60% to 80% of the DE provided by barley grain; Ferrer *et al.*, 2018) and that its inclusion up to 100 g/kg of OC in diets replacing barley grain on a weight basis does not impair feed intake and growth but decreases dietary DE concentration and carcass conformation and backfat thickness (Joven *et al.*, 2014). In addition, its use as a feed ingredient might modify slurry production, composition and gas emission (Ferrer *et al.*, 2018). These effects might be related to its high fibre and phenolic content. Previous research has shown that including fibrous by-products (e.g. sugar beet pulp, orange pulp, carob meal or rapeseed meal) in diets for growing pigs can help to reduce ammonia (NH₃) and occasionally methane (CH₄) emission from faeces or slurry, per unit of nitrogen (N) or organic matter (OM), respectively (Canh *et al.*, 1997; Torres-Pitarch *et al.*, 2014; Beccaccia *et al.*, 2015). In the case of OC its inclusion in diets might also decrease NH₃ emission from slurry (Ferrer *et al.*, 2018). On the other hand, olive by-products are rich in phenolic components (3.0 to 50.0 g/kg DM) with a high

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

The objective of the present study was to determine the effects of the inclusion of a partially defatted olive cake (PDOC) in balanced finishing pig diets on growth performance, carcass quality, faecal microbiology, slurry composition and gas emission.

Material and methods

Animals, diets and experimental design

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

Growth performance, carcass and meat quality

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

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
Ileal standardized 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		
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		
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		
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 using acid wet traps. A subsample from the exhausted air from the headspace of the tanks was forced to pass with an air pump through absorption flasks filled with 100 ml of 0.05N H₂SO₄. The quantity of total ammonia N (TAN) trapped in the absorption flasks was analysed following 4500 NH₃-D procedure (APHA, 2005) using a detection electrode (Orion High Performance NH₃ Electrode, model 9512HPBNWP, Thermo Scientific, USA).

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

Chemical analysis

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

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

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

The B₀ from the slurry was measured in a batch assay, using 120 ml glass bottles incubated at a mesophilic range (35°C \pm 1°C) for 100 days, following the methodology described by Ferrer *et al.* (2018). Anaerobic inoculum was collected from an anaerobic digester that treats domestic and industrial wastewater from the wastewater treatment plant in Sagunto (Spain), and pre-incubated for 15 days at 35°C in order to deplete the residual biodegradable organic material (degasification). An inoculum to substrate ratio of 1 on OM basis was used.

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

Statistical analysis

Data were analysed using SAS® (Statistical Analysis System) System Software (Version 9.1, SAS Institute Inc., Cary, NC, EEUU). Differences in BW, ADG, ADFI, FCR, BF, LD and carcass and meat quality traits between experimental treatments were tested by one-way ANOVA using the GLM procedure of SAS in a completely randomised block design, with the dietary treatment (C-diet and PDOC-diet) as the main effect and room as a block factor in the models. Microbial counts were log₁₀ transformed before analysis. For ADG, ADFI, FCR, initial and final weight, and microbial counts, the experimental unit was the pen, and for the carcass and meat quality measurements the individual pig was

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

Results

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

Partially defatted olive cake and experimental diets composition

The chemical composition of the OC used in the study is summarised in Tables 1 and 2. This OC presented a high EE (122 g/kg DM), sugar (82.2 g/kg DM) and DE (8.45 MJ/kg DM) content. Its fibre concentration was also high, particularly its ADL level. The analysed FA profile of OC revealed that oleic acid was the main FA (83.8 g/kg), followed by palmitic and linoleic acids. Regarding the chemical composition of the experimental diets (Tables 3 and 4), PDOC-diet showed a 39% higher EE, fibre (18% and 26% higher content of the NDF and ADF fractions), and lignin and polyphenol content compared with C-diet. The concentration of almost all FA (especially oleic acid) was also greater in the PDOC-diet compared with C-diet.

Growth performance, carcass and meat quality

The results on growth performance are summarised in Table 5. At the end of the study, BW was not significantly different between treatments. No significant differences were obtained in ADG or in ADFI. However, FCR tended to be higher (0.12 units; $P = 0.059$), and LD was significantly lower (2.02 units, $P = 0.02$) in the group of animals offered PDOC-diet. The carcass and meat quality traits measured are shown in Table 6. The inclusion of PDOC in pig diets had no significant effect on carcass characteristics, except the pH that was lower ($P < 0.001$) and the red colour (a^*) that tended ($P = 0.086$) to be lower in the meat of pigs offered PDOC-diet. Regarding the FA profile of the subcutaneous fat (Table 7), total MUFA concentration was higher and total PUFA concentration lower in the fat tissue of animals offered the diet with 12% PDOC compared with that of the C-diet ($P < 0.001$). The ratio MUFA/SFA was higher and the ratio PUFA/SFA lower in the pigs offered PDOC compared with the pigs offered C-diets ($P < 0.05$). Taking into account individual FA, the biggest differences between treatments were found for the MUFA acids, especially palmitoleic, heptadecenoic, oleic and vaccenic acids ($P < 0.05$), in the fat of animals 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 (<i>L</i> [*])	37.4	37.4	0.400	0.979
Redness (<i>a</i> [*])	8.76	8.10	0.277	0.086
Yellowness (<i>b</i> [*])	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.

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

Microbial counts

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

Slurry composition and gas emission

The amount of slurry produced by the animals offered PDOC 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 composition, the ADF concentration in the slurry of animals offered PDOC was significantly higher than that of the animals offered C-diet ($P < 0.05$). In addition, ADL concentration in the slurry tended to be higher ($P = 0.06$), and the EE concentration in the slurry tended to be lower ($P = 0.08$) in the group of animals offered PDOC. Concerning the gas emissions, no significant differences were obtained either on the B_0 values or on the amount of gas emitted expressed per litre of slurry or per animal and day.

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
Emission per tank, 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.

Discussion

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

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

The inclusion of fibrous by-products in pig diets, such as PDOC, has been related to poorer performance traits especially during the growing phase (Jarrett and Ashworth, 2018). However, in the current study, diets were formulated to be isonutritive and, accordingly, the inclusion of a 12% PDOC did not lead to significant differences in growth performance, although a trend to a slight decrease of feed efficiency (FCR 0.12 units higher; $P = 0.06$) was detected in pigs offered PDOC. This might indicate that the net energy value for PDOC was overestimated in the current study, since pigs offered PDOC-diet increased feed consumption to meet their energy requirements. On the other hand, *in vivo* BF measurement was similar between treatments, but LD was about 2 mm lower ($P < 0.05$) on average in the group of pigs fed PDOC-diet. Joven *et al.* (2014) also showed a linear decrease of fat depots in the carcass as the level of OC increased from 0% to 15% in finishing pigs. Mas *et al.* (2010) and González *et al.* (2012) described no differences in carcass characteristics from pigs offered diets enriched with oleic acid and olive pomace oil (65 g/kg), respectively. Differences in carcass fat content are expected when pigs consume more energy than required with high-energy diets or when the indispensable amino acid or protein levels in diets are lower than required (Cámara *et al.* 2016). With respect to meat quality traits, Serra *et al.* (2018) showed similar pH levels and lower yellowness (b^*) with the inclusion of olive pomace in pig diets. Despite this our results are in accordance with those authors, only the pH values were affected by feeding OC. Pigs fed OC showed a lower meat pH that could be associated with higher muscle glycogen stores. Although a statistical difference has been observed in the muscle pH at 2 h *postmortem*, it has no practical implications on quality traits (no differences were found in colour parameters). Besides, pH values in the range of 6.6 to 6.0 after 2 h *postmortem* are not associated with the development of pale, soft and exudative (PSE) or dark, firm and dry (DFD) meats (Rosenvold and Andersen, 2003).

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


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

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

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

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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 significant 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 (registration number 2016/VSC/PEA/00024).

Software and data repository resources

None of the data were deposited in an official repository.

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