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RESEARCH ARTICLE

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Ensiled citrus pulp as a by-product feedstuff for finishing pigs: nutritional value and effects on intestinal microflora and carcass quality

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Abstract

Forty pigs of 76.8 ± 4.2 kg body weight were fed four different diets varying in ensiled citrus pulp (ECP) inclusion level (0, 50, 100, and 150 g of ECP/kg of diet on dry matter base). The trial lasted 5 weeks. During the last week, faecal samples were obtained to calculate apparent nutrient digestibility; also volatile fatty acids (VFA) content in faeces was determined. The digestible energy and protein of ECP was estimated by regression analysis. During the trial faecal samples were collected to determine enterobacteria and lactobacilli counts. At slaughter, carcass characteristics were registered. The inclusion of ECP in the diets decreased energy digestibility but increased neutral and acid detergent fibre digestibility linearly ($p<0.05$). The estimated digestible energy and protein of ECP were lower than expected (7.0 MJ/kg dry matter (DM) and 33.8 g/kg DM, respectively). Total VFA production in faeces was not affected by the diet. Both enterobacteria and lactobacilli counts were lower ($p<0.01$) with than without ECP inclusion at the end of the study. Carcass yield decreased linearly ($p<0.05$) and backfat at *gluteus medius* increased quadratically ($p<0.05$) with the inclusion of ECP in the diet. The polyunsaturated fatty acid content of the internal subcutaneous fat layer decreased quadratically ($p<0.05$) with the inclusion of ECP. Thus, the nutritive value of ECP for finishing pigs is low and levels of 150g/kg can negatively affect carcass yield. However, including up to 100 g of ECP/kg in finishing pig diets did not affect nutrient digestibility, carcass yield and subcutaneous fat FA profile. Additionally, increasing ECP levels in diets decreases faecal enterobacteria and lactobacilli counts in faeces.

Additional key words: agroindustrial by-products; digestibility; gut microbiology; carcass performance.

Abbreviations used: ADF (acid detergent fibre); ADFI (average daily feed intake); ADG (average daily gain); ADL (acid detergent lignin); BW (body weight); CFU (colony forming units); CP (crude protein); CTTAD (coefficient of total tract apparent digestibility); DM (dry matter); ECP (ensiled citrus pulp); EE (ether extract); FA (fatty acid); GE (gross energy); L (linear); Lys (lysine); NDF (neutral detergent fibre); NDSF (neutral detergent soluble fibre); OM (organic matter); Q (quadratic); VFA (volatile fatty acid).

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Introduction

The use of non-conventional feed resources such as food industry by-products that can replace cereals in livestock diets is becoming increasingly important as a consequence of the high demand of cereals for industrial purposes. Additionally, the use of alternative feedstuffs in livestock diets decreases competitiveness of animals' feeding with human feeding, an issue of global relevance since many countries in the world base their diets on cereals.

By-products of the citrus juice industry, which are primarily used in ruminants, might also be used as an alternative source of energy in fattening pigs, becoming an opportunity to decrease feed costs and grain dependence also in the swine sector. Among the citrus by-products most commonly used in animal feeding are whole citrus fruits not valid to be commercialized as food and citrus pulp. Citrus pulp, which is the whole residue after the juice extraction, is the major worldwide citrus by-product. It contains the peel, the rag and the seeds of the original fruit. This can be commercialized in fresh or in dry form and shows a lower moisture, sugar and pectin content than whole citrus fruits. Citrus by-products can be composed by a unique sort of citrus (oranges, mandarins, lemons and grapefruits) or a combination of them. This confers certain variability in terms of composition to these by-products. For example, lemons contain a high fibre and low sugar content compared with mandarins and oranges (Piquer, 2006). In general, the moisture content of citrus pulp is greater than 800 g/kg (Martínez-Pascual & Fernández-Carmona, 1980) and it has a high content in soluble fibre, including pectin (220 g/kg dry matter (DM); Bampidis & Robinson, 2006). This makes it suitable for inclusion in ruminant diets as a source of energy (Bampidis & Robinson, 2006; Fung *et al.*, 2010); however, little is known on its acceptability and nutritive value for pigs. Although pigs exhibit a low potential for fibre utilization, the digestibility coefficient of fibre sources high in pectin or water-soluble components such as citrus pulp in heavy pigs can range from 0.70 to 0.90 (Noblet & Le Goff, 2001). Accordingly, the suggested digestible energy contents for dried citrus pulp in pigs range from 9.6 to 12.1 MJ/kg DM (De Mol, 1992). However, maximum levels of inclusion in pig diets are unclear. While some studies do not recommend levels above 50 or 100 g/kg DM diet due to reductions in growth performance and carcass quality (Baird *et al.,* 1974; O'Sullivan *et al.,* 2003; Watanabe *et al*., 2010a,b; Crosswhite *et al*., 2013), others demonstrate no negative effects on performance (Amorim *et al*., 2014) and meat quality (Watanabe *et al*., 2010b) at higher levels of inclusion.

In high producing areas, these by-products are a major concern and its use in animal feeding contributes to alleviate the environmental burdens linked to its elimination. In those areas, citrus pulp is usually commercialized fresh or ensiled, since drying is not cost-effective. The direct use of forages or fresh products in pig feeding has been mainly developed in tropical countries such as Vietnam, Colombia or Guadeloupe but also in United States and European countries such as The Netherlands (Rijnen *et al.,* 2001; Leterme *et al.,* 2005; Xandé *et al.,* 2010; Crosswhite *et al*., 2013) although its use is still limited in pigs. Ensiling implies a fermentation process that might improve palatability and reduce microbial contamination (Kayouli & Lee, 1998), but also can change the nutritional value of the raw material. A previous study (Cerisuelo *et al.,* 2010) reported that the inclusion of 100 g ensiled citrus pulp (ECP)/kg in growing pigs did not affect growth performance after an adaptation period of four weeks and some benefits on gut microbiology were observed. Therefore, ECP might be used at some extent in pig feeding, although information on its nutritive value for pigs is limited.

The aim of the present study was to evaluate the effects of including up to 150 g/kg of ensiled citrus pulp in finishing pig diets on nutrient digestibility and to estimate its energy and digestible protein content and its effects on gut microbiology and carcass quality.

Material and methods

Experimental design

The experimental procedures were approved by the Instituto Valenciano de Investigaciones Agrarias (Moncada, Valencia) Animal Ethics Committee and fulfilled the European regulations for the Care and Use of Animals for Scientific Purposes. Forty Pietrain × (Landrace × Large White) finishing pigs (20 males and 20 females) were randomly selected at 76.8 ± 4.19 kg body weight (BW) and allocated individually in 3 × 3 m pens. Four experimental diets were formulated to contain different levels of ECP on a DM basis: 0 (basal diet), 50, 100, and 150 g/kg of diet. To obtain ECP, the original fresh citrus pulp obtained from a single juice citrus industry was ensiled in a trench silo for 20 days. This citrus pulp contained only orange wastes.

The basal diet consisted in a commercial pelleted diet for finishing pigs. The composition of the basal diet used in this study is shown in Table 1. The diets with 50, 100, and 150 g of ECP/kg (dry matter basis) were manufactured every 2 days by mixing the basal diet with the corresponding amount of ECP. Additionally, to reduce the differences in terms of crude protein (CP) and lysine (Lys) content among diets caused by the addition of ECP, different amounts of soybean meal and L-lysine were added to the diets containing ECP. Concretely, per kilogram of diet we added 28, 40 and 50 g of soybean meal and 0, 0.5 and 1 g of lysine in diets including 50, 100 and 150 g ECP/kg, respectively. All the ingredients (basal diet, citrus pulp, additional soybean meal and crystalline Lys) were mixed by using an automatic mixer of 150 L capacity in order to obtain a homogeneous mixture. Calculated and analysed nutrient concentration of the experimental diets and the ECP is shown in Table 2.

The trial lasted 5 weeks (experimental period) in which pigs were fed the experimental diets. At the beginning of the experimental period pigs weighed 91.5 ± 6.09 kg BW. Before the start of the trial (pre-experimental period), pigs were allowed one week to become

Table 1. Ingredient composition of the basal diet (g/kg, as feed basis)

Ingredients	Basal diet
Wheat	390
Barley	127
Rice bran	100
Rapeseed meal	90.0
Soybean meal, 47% CP	76.0
Maize	68.0
Hominy meal	54.5
Sunflower meal	27.0
Lard	27.0
Beet molasses	10.0
L-Lysine HCl	6.33
Threonine	1.18
Methionine	0.99
Calcium carbonate	11.0
Salt	4.00
Liquid acid[1]	2.00
Vitamin-mineral premix[2]	5.00

[1]The composition of the liquid acid is 75% formic acid and 25% lactic acid. [2]Vitamin-mineral premix contains, as supplied per kg of diet: vitamin A, 7,500 I.U.; vitamin D3, 2,000 I.U.; vitamin E, 15 mg; vitamin K3 (menadione), 0.50 mg; vitamin B1, 1 mg; vitamin B2, 3 mg; vitamin B6, 1 mg; vitamin B12, 0.02 mg; nicotinic acid, 15 mg; pantothenic acid, 9.9 mg; iron, 80 mg; copper, 20 mg; cobalt, 0.31 mg; zinc, 100 mg; manganese, 43.2 mg; iodine, 1.8 mg; selenium, 0.18 mg.

accustomed to the pens (week –2) and one week to get adapted to the smell, texture and taste of ECP itself (week –1). During the week of adaptation to ECP (week –1), this was provided as free choice (separated from the feed) and *ad libitum* in the groups of pigs that were going to be fed the diets containing ECP. A fixed amount of 2.70 kg DM feed/d (2.5 times approximately maintenance requirement for net energy considering the energy content of the basal diet; NRC, 2012) was provided in two equal doses at 08:00 h and 12:00 h during all the experimental period. Water was provided *ad libitum* throughout the study. At the end of the study (125 ± 7.6 kg BW) all animals were slaughtered and carcass characteristics were recorded.

Measurements

Pigs were weighed at allocation, at the beginning of the experimental period and the day before slaughter. The digestibility balance was performed on the last week of the experimental period using only males (n = 20) and males were also weighed 2 days prior the digestibility balance. The coefficient of total tract apparent digestibility (CTTAD) of nutrients was determined by recording average daily feed intake (ADFI) and total production of faeces for three consecutive days using the Velcro bag methodology (Van Kleef *et al.,* 1994). A representative sample of each diet

Table 2. Analysed and calculated chemical composition (g/kg dry matter [DM] unless otherwise indicated) of ensiled citrus pulp and experimental diets containing different amount of ensiled citrus pulp fed to finishing pigs

	Ensiled citrus pulp	Ensiled citrus pulp content in experimental diets [g/kg DM]			
		0	50	100	150
Dry matter	155	895	688	547	480
Analyzed composition					
Gross energy (MJ/kg DM)	–	19.7	19.8	19.5	18.6
Crude protein (N × 6.25)	90	155	160	165	170
Crude fat	32	–	–	–	–
Neutral detergent fibre	301	197	205	210	220
Acid detergent fibre	198	76.4	85.6	92.5	100.6
Acid detergent lignin	0	15.5	15.8	13.2	13.7
Neutral detergent soluble fibre	356				
Ash	74	73.3	71.5	73.6	74.2
Calculated composition †					
Metabolizable energy (MJ/kg DM)	–	14.8	14.8	14.5	14.2
Crude fat	–	80.6	68.7	66.1	63.5
Lysine	–	10.9	10.9	10.9	11.0
Methionine + Cysteine	–	7.26	7.10	6.82	6.52
Threonine	–	7.40	7.34	7.10	6.84

† Calculated nutrient composition is based on FEDNA (2010).

(1,000 g) was collected daily during the digestibility balance and stored at -20ºC until subsequent analyses. Faeces produced by each individual pig were weighed and homogenized every 24 h, and a sample (300 g) was stored at –20ºC until subsequent analyses of nutrient and volatile fatty acids (VFA).

In addition, faecal samples were aseptically removed directly from the rectum of 8 animals (4 males and 4 females) per treatment before the pre-experimental period (week –3) and on weeks +2 and +5 of the experimental period for bacterial (enterobacteria and lactobacilli) enumeration. These bacterial populations were used as indicators of gut health with a low number of *Enterobacteriaceae* and high number of lactobacilli being considered beneficial (Canibe & Jensen, 2003; Ewing, 2008; Ivarsson *et al.,* 2012).

At the end of the study, pigs were fasted for approximately 20 h and slaughtered. On the day of slaughter, animals were transported from the pig house to the slaughterhouse and rested for a minimum of 1 h before slaughter. The pigs were stunned by CO_2, exsanguinated, scalded, cleaned, and eviscerated within ~30 min. After 2 h of sacrifice, the carcasses were placed at 4°C in a chilling room for 24 h. Carcass weight was recorded immediately after slaughter (hot carcass weight). At approximately 2 h *post-mortem* (at the chilling room), fat depth was measured with a metal ruler at the level of the *gluteus medius* in the left part of the carcass. Also at this time, a sample of subcutaneous fat was removed at the level of the second cervical vertebrae and the fatty acid (FA) profile of the two fat layers was determined by gas chromatography (O'Fallon *et al.,* 2007). The experimental unit was the individual pig for all variables.

Chemical analyses

Before analysis, feed and faecal samples obtained during the digestibility trial were pooled across days for each pig. Feed samples were analysed for DM, ash and CP following AOAC (2000) procedures. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) content were analysed sequentially with a thermo-stable amylase pre-treatment (Van Soest *et al.,* 1991). Gross energy (GE) was determined using adiabatic calorimetry (Gallenkamp Autobomb; Loughborough, UK). Composition of ECP was analysed before the start of the trial for DM, CP and ether extract (EE) using the method outlined by AOAC (2000), and for NDF and ADF as indicated for the diets. Additionally, the neutral detergent soluble fibre (NDSF) content of ECP was analysed using a modification of the methodology proposed by Hall *et al.* (1997) described by Martínez-Vallespín *et al.* (2011). Briefly, NDSF includes soluble and insoluble pectins, soluble hemicelluloses (arabinoxylans and β-glucans), fructans and oligosaccharides and was calculated as the difference between the organic matter (OM) fraction and the starch, protein, and NDF (corrected by CP content) fractions, all included in the ether-ethanol insoluble residue. Faeces were analysed for DM, ash, CP, GE, NDF and ADF as indicated for the diets. Additionally, the volatile fatty acid (VFA) concentration in the faeces collected in the digestibility trial was analysed by gas chromatography, with the addition of 4-metil valeric as internal standard (Jouany, 1982).

Immediately after collection, faecal samples for bacterial counts were diluted 1:10 (1 g faeces in 9 mL of peptone water) and decimal dilutions of each sample were prepared. The number of colony forming units (CFU) of enterobacteria and lactobacilli were counted by plating 0.1 mL of each dilution in selective media. Enterobacteria were isolated on McConkey agar (Liofichem, Roseto degli Abruzzi, Teramo, Italy), following aerobic incubation at 37ºC for 24 h. Lactobacilli were cultured on Man, Rogosa, Sharp agar (MRS, Liofilchem, Roseto degli Abruzzi, Teramo, Italy) following incubation at 30ºC for 72 h. All colonies on the MRS plates were counted as lactobacilli.

Calculation and statistical analysis

Data were analyzed using SAS System Software (SAS Inst. Inc., Cary, NC, USA). Differences in BW, ADFI, CTTAD, VFA content in faeces and carcass quality traits among experimental treatments were tested by analysis of variance using the GLM procedure of SAS, and applying polynomial orthogonal contrasts to test linear (L) and quadratic (Q) effects. Changes in bacterial counts with time were analysed according to a repeated measures model (MIXED procedure of SAS). Enterobacteria and lactobacilli counts were $\log_{10}$ transformed before analysis. In all cases, the individual pig was considered the experimental unit and the treatment was considered the main factor in the models. For carcass quality traits analyses, sex was additionally included as a block factor in the model.

The CTTAD of OM, CP, GE, NDF and ADF for the different experimental diets was calculated. The digestible energy and digestible protein content of ECP was then estimated by extrapolation to a total substitution (100%) using lineal regression (REG procedure of SAS) between the digestible nutrient content of experimental diets and the substitution rates, following the methodology proposed by Villamide *et al.* (2003).

This method was chosen because, as Villamide *et al.* (2003) suggested, this is a reliable method for imbalanced feedstuffs since it is based on the effect of the test ingredient in several diets and it does not only depend on only one diet that could have an unusual digestive behaviour. The digestible energy and protein of the extra soybean meal and Lys added to diets with ECP was estimated (14.1 MJ/kg of DM and 451 g/kg of DM for the soybean meal and 20.8 MJ/kg of DM and 964 g/kg of DM for the Lys, respectively; De Blas *et al.,* 2010) and subtracted. The digestibility values of the diet containing 150 g of ECP/kg imbalanced the regression model in the case of energy. Thus, digestibility values of this experimental diet were not considered representative and they were eliminated from the model.

Results

Feeds composition

The DM content of the diets decreased from 895 g/kg to 480 g/kg and the gross energy (GE) content decreased from 19.7 to 18.6 MJ/kg of DM as the level of ECP increased from 0 to 150 g/kg of diet (Table 2). In terms of amino acids, the Lys level was similar among diets but the Met+Cys and Thr levels decreased with the inclusion of ECP. Neutral detergent fibre and ADF increased with the inclusion of ECP (NDF: from 197 to 220 g/kg and ADF: from 76 to 101 g/kg).

Performance and apparent digestibility of diets and ECP

Male pigs weighed 122 ± 8.5 kg two days before the digestibility trial and feed intake expressed in DM was similar among groups of treatment (Table 3). Additionally, average daily gain (ADG) from males from the beginning of the experimental period to the end of the study was similar among treatments. The CTTAD of OM and CP was not different among treatments (Table 3). However, GE digestibility was reduced as the level of ECP in the diet increased following a L effect ($p<0.05$). The CTTAD of NDF and ADF increased with the inclusion of ECP in the diet, both following a significant ($p<0.05$) L effect. The DM content of faeces decreased significantly ($p<0.001$) as the inclusion of ECP in the diet increased being 292, 289, 277 and 265 g/kg for the animals fed the diets with 0, 50, 100 and 150 g ECP/kg, respectively (data not shown in tables). Following the regression equations obtained to calculate the amount of digestible energy and digestible protein in ECP (digestible energy [MJ/kg of DM] = 15.6 – 8.64x, $R^2 = 0.56$ and digestible protein [g/kg of DM] = 120.1 – 86.34x, $R^2 = 0.66$), the ECP contained on DM basis 7.0 MJ/kg of digestible energy and 37.7 g/kg of digestible protein. In the case of digestible energy, as previously indicated, ECP inclusion at levels higher than 100 g/kg in pig diets affected the digestibility of energy and nutrients of the basal diet and imbalanced the model in the present study. For this reason this treatment was eliminated from the prediction model.

Table 3. Effect of feeding diets containing ensiled citrus pulp on performance and coefficient of apparent faecal digestibility of nutrients in male finishing pigs

Item	Ensiled citrus pulp content in experimental diets (g/kg DM)					Statistical analyses	
	0	50	100	150	Pooled SE	Linear effect	Quadratic effect
Number of pigs	5	5	5	5	–	–	–
Pig's weight (kg)[1]	125	121	122	121	4.1	NS	NS
Feed intake (kg DM/d)[2]	2.66	2.52	2.65	2.45	0.075	NS	NS
ADG (kg/d)[3]	0.870	0.780	0.774	0.874	0.050	NS	NS
Coefficient of apparent total tract digestibility (%)							
Organic matter	79.9	79.8	79.5	78.2	0.8	0.099	NS
Gross energy	79.3^{a}	78.5a	78.0a	75.0b	1.0	**	NS
Crude protein	78.7	78.2	77.4	76.6	1.2	NS	NS
Neutral detergent fibre	43.0^{b}	47.5^{ab}	49.6^{a}	49.8^{a}	2.4	*	NS
Acid detergent fibre	18.2^{c}	28.3^{b}	34.9^{ab}	38.1^{a}	3.2	***	NS

[1] Pigs were weighed two days before the digestibility trial. [2] Feed intake was recorded during the digestibility balance. [3] Average daily gain was recorded throughout the experimental period. DM, dry matter; SE, standard error; NS, non-significant. Means along rows for each effect not sharing the same superscript are significantly different ($p<0.05$). Levels of significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Volatile fatty acids and gut microbiology

The concentration and proportions of total VFA in faeces are presented in Table 4. As the Q effects were not significant these were not shown in this table. VFA are considered indicative of the total production and proportion in the gut (Williams *et al.,* 2001). Total VFA content in faeces was not affected by the inclusion of ECP in the diet. Relative to total VFA concentration, acetic acid was the main VFA contributing to total faecal VFA in all diets, but especially in pigs fed with 150 g ECP/kg of diet. The contribution of acetic acid to total VFA in this group of pigs was significantly higher ($p<0.05$) compared to the rest of treatments, following a clear L effect among treatments ($p<0.01$). On the contrary, the contribution of the rest of VFA especially propionic and valeric acids to total VFA was higher in the group of pigs fed the basal diet, also following a significant L effect among treatments ($p<0.05$).

As regards to bacterial counts in faeces, the repeated measures analysis performed showed a significant effect of diet and week for enterobacteria and lactobacilli counts ($p<0.01$). Enterobacteria and lactobacilli counts before the pre-experimental period (week –3) were similar among treatments. On week +2 of the experimental period, pigs fed 150 g ECP/kg had the lowest enterobacteria counts (Table 5, $p<0.05$) compared to the

Table 4. Effect of feeding diets containing ensiled citrus pulp on faecal volatile fatty acid (VFA) content in finishing pigs

Item	Ensiled citrus pulp content in experimental diets (g/kg DM)					Statistical analyses (Linear effect)
	0	50	100	150	Pooled SE	
Number of pigs	5	5	5	5	–	–
Total VFA (mmol/g)	164	179	174	175	8.3	NS
Proportion of individual VFA relative to total VFA						
Acetic acid	0.60^{b}	0.60^{b}	0.61^{ab}	0.64^{a}	0.010	**
Propionic acid	0.21^{a}	0.21^{ab}	0.20^{ab}	0.20^{b}	0.005	*
Isobutyric acid	0.02	0.02	0.02	0.02	0.001	0.095
Butyric acid	0.12	0.12	0.12	0.11	0.006	NS
Isovaleric acid	0.03	0.03	0.03	0.03	0.002	0.087
Valeric acid	0.02^{a}	0.02^{ab}	0.02^{ab}	0.01^{b}	0.001	*

SE, standard error; NS, non-significant. Means along rows for each effect not sharing the same superscript are significantly different ($p<0.05$). Levels of significance: *$p<0.05$, $^{**}p<0.01$, ***$p<0.001$.

Table 5. Effect of feeding diets containing ensiled citrus pulp on faecal bacteria (enterobacteria and lactobacilli) counts in finishing pigs

Item	Time	Ensiled citrus pulp content in experimental diets (g/kg DM)				
		0	50	100	150	Pooled SE
Number of pigs		8	8	8	8	–
Enterobacteria counts (Log$_{10}$ CFU/g faeces)	Week –3[†]	7.16	7.19	6.97	6.73	0.25
	Week +2	7.23^{a}	6.95^{a}	6.85^{a}	6.58^{b}	0.25
	Week +5	7.10^{a}	6.57^{b}	6.11^{b}	6.15^{b}	0.22
Lactobacilli counts (Log$_{10}$ CFU/g faeces)	Week –3[†]	8.52	8.61	8.41	8.47	0.16
	Week +2	9.09^{a}	8.89^{a}	8.45^{b}	8.64^{ab}	0.15
	Week +5	9.01^{a}	8.53^{ab}	8.33^{bc}	7.97^{c}	0.19
Ratio Lactobacilli:Enterobacteria	Week –3[†]	1.21	1.21	1.22	1.27	0.05
	Week +2	1.27	1.30	1.24	1.35	0.06
	Week +5	1.28	1.35	1.37	1.31	0.05

[†] Before the pre-experimental period (before a two-week adaptation period to the pen and to the ensiled citrus pulp). DM, dry matter; SE, standard error; CFU: colony-forming units. Means along rows for each effect not sharing the same superscript are significantly different ($p<0.05$). The p values for diet, week and diet × week given by the statistical procedure (proc MIXED) were <0.01, <0.01 and NS for enterobacteria counts, <0.001, <0.01 and 0.06 for lactobacilli counts and NS, <0.01 and NS for the ratio lactobacilli:enterobacteria, respectively.

other groups of pigs and at the end of the trial (week +5), all the groups fed with ECP showed significantly lower enterobacteria counts compared to the pigs fed the basal diet ($p<0.05$). Lactobacilli counts also decreased with the inclusion of ECP in diet on weeks +2 and +5 of the experimental period ($p<0.05$). Consequently, the ratio lactobacilli to enterobacteria was not different among treatments throughout the study.

Carcass and meat quality

Carcass weight was not affected by the diet (Table 6). However, carcass yield and fat depth at the *gluteus medius* level were reduced by ECP following a L ($p<0.001$) and a Q ($p<0.05$) relationship, respectively. For fat depth, the maximum levels were detected in the group of pigs fed with 50 and 100 g ECP/kg of feed and

Table 6. Effect of feeding diets containing ensiled citrus pulp on carcass quality traits and fatty acid profile of the subcutaneous fat in finishing pigs

Item	Ensiled citrus pulp content in experimental diets (g/kg DM)					Statistical analyses	
	0	50	100	150	Pooled SE	Linear effect	Quadratic effect
Number of pigs	10	10	10	10	–	–	–
Final body weight (kg)	126.2	123.8	125.1	125.7	1.35	NS	NS
Carcass weight (kg)	93.9	92.4	92.5	91.9	2.4	NS	NS
Carcass yield (%)	74.6^{a}	74.0^{a}	73.7^{ab}	72.6^{b}	0.5	***	NS
Fat depth at *gluteus medius* (mm)	9.6	12.8	12.3	10.0	1.4	NS	*
Fatty acid profile of subcutaneous fat[1] (%)							
—External layer							
Saturated	30.2	30.7	30.7	31.2	0.6	NS	NS
C14:0	0.87	0.92	0.86	0.88	0.031	NS	NS
C16:0	20.8	20.8	20.8	21.0	0.317	NS	NS
C18:0	8.59	9.02	8.99	9.33	0.301	NS	NS
Monounsaturated	52.2	52.6	52.4	51.3	0.7	NS	NS
C17:1	0.38	0.39	0.38	0.36	0.021	NS	NS
C16:1	2.35	2.31	2.27	2.15	0.083	NS	NS
C18:1	49.4	49.8	49.9	48.7	0.744	NS	NS
Polyunsaturated	17.6	16.6	16.9	17.5	0.5	NS	NS
C18:2	16.7	15.7	15.7	16.6	0.637	NS	NS
C18:3	0.94	0.99	1.02	1.02	0.030	*	NS
Ratio[2]	2.32	2.26	2.27	2.21	0.06	NS	NS
—Internal layer							
Saturated	31.6	31.9	32.4	32.7	0.6	NS	NS
C14:0	0.88	0.83	0.93	0.90	0.062	NS	NS
C16:0	20.1	20.3	20.5	20.6	0.282	NS	NS
C18:0	10.7	10.7	10.9	11.3	0.434	NS	NS
Monounsaturated	48.7^{ab}	49.8^{a}	49.4^{ab}	48.4^{b}	0.5	NS	*
C17:1	0.38	0.40	0.39	0.36	0.019	NS	NS
C16:1	1.94	2.10	2.07	1.95	0.069	NS	*
C18:1	45.5	46.4	46.2	45.2	0.566	NS	*
Polyunsaturated	19.7^{a}	18.3^{b}	18.2^{b}	18.9^{ab}	0.5	NS	*
C18:2	17.7	16.4	16.2	16.8	0.519	NS	*
C18:3	0.94	0.96	0.94	0.98	0.029	NS	NS
Ratio[2]	2.17	2.14	2.10	2.06	0.06	NS	NS

[1] Saturated fatty acids include C14:0, C16:0 and C18:0; monounsaturated fatty acids include C17:1, C16:1 and C18:1; polyunsaturated fatty acids include C18:2 and C18:3; unsaturated:saturated ratio considers (monounsaturated + polyunsaturated):saturated. The main fatty acids of each group are also shown. [2]Unsaturated:saturated ratio. Means along rows for each effect not sharing the same superscript are significantly different ($p<0.05$). Levels of significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

the minimum in the group of pigs fed the basal diet. The FA profile in the external layer of the subcutaneous fat was similar for all treatments, except for C18:3 (alpha-linolenic acid) which increased linearly with ECP inclusion. In all groups, the monounsaturated acids were the most important fraction of FA in this layer. In the internal subcutaneous fat layer, the monounsaturated was also the main fraction obtained. Among treatments, monounsaturated FA (especially C16:1, palmitoleic acid, and C18:1, oleic acid) increased and polyunsaturated FA (especially C18:2, alpha-linoleic acid) decreased quadratically ($p<0.05$) with ECP inclusion. The sex effect was also tested for these parameters, although the results are not shown in tables. Sex effect was significant ($p<0.05$) for fat depth at *gluteus medius* level and the monounsaturated and polyunsaturated FA. Females showed a greater fat depth level at *gluteus medius* and a greater percentage of monounsaturated FA and a lower percentage of polyunsaturated FA than males. However, the interaction diet × sex was not significant.

Discussion

Digestibility and performance

Ensiled citrus pulp is proposed as an alternative feedstuff for pig nutrition in this study. Its inclusion at levels up to 150 g/kg in finishing pig diets showed effects on nutrient digestibility reducing GE and increasing NDF- and ADF-CTTAD; however, the digestibility of GE was acceptable up to 100 g inclusion/kg of diet. These results are in agreement with those reported in the literature (Baird *et al.,* 1974; Galassi *et al.,* 2004; Watanabe *et al*., 2010a; Ruiz *et al*., 2012) in which the inclusion of dried citrus pulp or other pectin-rich feedstuff such as sugar beet pulp lowered nitrogen (N) and EE digestibility but significantly increased fibre digestibility. Also Rijnen *et al.* (2001) found increased fibre digestion in sows fed diets with sugar beet pulp silage. The fibre fraction of the ECP in this study was highly digestible and above that of other more lignified feed ingredients, which is due to its high pectin and soluble fibre content (Watanabe *et al*., 2010a). In fact, the NDSF fraction determined in this study for ECP was relatively high compared to that given for other feedstuffs such as sorghum DDGS (Cerisuelo *et al.,* 2012) and corn DDGS (Stein & Shurson, 2009).

Typically, an excess of dietary fibre in pig diets, especially fermentable fibre, reduces feed intake (Kyriazakis & Emmans, 1995; Bindelle *et al.,* 2008) due to the high water holding capacity of specific non-starch polysaccharides such as pectins. In the current study, ADFI was measured only in males during the digestibility trial and no significant differences were observed in terms of DM intake among treatments. Cerisuelo *et al.* (2010) also observed that after 3-4 weeks of feeding diets with ECP feed consumption stabilized. Accordingly, ADG did not differ among treatments. Other studies in the literature reported no differences in feed intake and weight gain when including up to 150 g/kg dried citrus pulp in pig diets (Amorim *et al*., 2014). However, Crosswhite *et al.* (2013) found a reduction in DM feed intake when levels of 150 g/kg (DM basis) of ECP were included in pig diets. According to Cerisuelo *et al.* (2010), the short (7 days) acclimation period followed in this study could partially explain this decrease in feed intake. On the other hand, it must be taken into account, that due to the low number of replicates (5 pigs per treatment) in the present study, data on ADFI in the present study should be carefully interpreted.

Digestible energy and digestible protein of ECP

The digestible energy values for dried citrus pulp in pigs reported in the literature range from 9.6 to 12.1 MJ/kg of DM (De Mol, 1992). O'Sullivan *et al.* (2003) estimated the digestible energy value of dried citrus pulp for growing pigs to be 12.0 MJ/kg of DM. Watanabe *et al*. (2010a) reported digestible energy values for dried citrus pulp to be around 10.4 MJ/kg of DM). Both values are lower than those reported for most cereals used in animal nutrition. So far, no research has been published on the digestible energy content of ECP. In the present study, ECP had a low digestible energy content (7.0 MJ/kg DM), although higher than the estimated for other fibrous feedstuffs such as oat hulls, olive pulp or grape pulp, and comparable to those of alfalfa hay, soybean hulls and other tropical tree foliage (Leterme *et al.,* 2005; De Blas *et al.,* 2010). One of the reasons that could explain this low energetic value of ECP as compared with fresh or dried citrus pulp is a change in chemical composition during the ensiling process. Unpublished data from our group indicates that total soluble sugar content of fresh citrus pulp decreased from 354 to 122 g/kg and NDF content increased from 188 to 301 g/kg during the ensiling process (20 days). Additionally, Cervera *et al.* (1985) found that water soluble carbohydrates disappeared quickly during the first 10 d of the ensiling process of citrus pulp. In terms of antinutritional factors, Oluremi *et al.* (2010) reported that the concentration of phytonutrients such as oxalate, flavonoid, tannin, saponin and phytate in citrus fruit peels were below the levels reported to be toxic to livestock species and that fermentation caused a reduction in its concentration. Thus, it seems improbable that the presence of antinutritional factors in ECP can affect its nutritive value. Information on the digestible protein content of citrus by-products in pigs is also scarce in the literature. In the present study, digestible protein of ECP was low (33.8 g/kg of DM), but this was expected since the mean CP content of citrus by-products is generally low and often linked to fibre (Gasa *et al.,* 1992). On the other hand, Watanabe *et al*. (2010a) reported digestible protein contents even lower for dried citrus pulp (8.9 g/kg of DM), thus indicating that the ensiling process could increase the availability of the inherent low digestible protein of fruits. Overall, in terms of nutritive value, ECP seems a low valuable feedstuff in pig nutrition. As suggested by Noblet & Le Goff (2001), although pigs are able to digest dietary fibre to a reasonable extent, the net supply of energy from fibre digestion can be low. Thus, the use of fibrous ingredients in pig diets as an alternative to cereals may not always be efficient in terms of nutrient contribution. However, as for other by-products, the economical asset of its use in pig production must be also considered.

Volatile fatty acids and gut microbiology

VFA (mainly acetate, propionate and butyrate) from dietary fibre fermentation contribute to maintain gut integrity and also provide energy to the gut and the whole body of non-ruminants (Anguita *et al.,* 2006). Its production and proportion may change according to diet composition. In the present study, VFA production was not measured but VFA content in faeces was similar among treatments. In terms of VFA proportion, the inclusion of 150 g of ECP/kg of DM increased the proportion of acetate ($p<0.05$), reaching levels of 0.64 in pigs fed diets with 150 g ECP/kg and decreased the proportion of other acids, especially propionic and valeric acids in faeces. Other studies (Zacharias *et al.,* 2004; Anguita *et al.,* 2006; Fung *et al.*, 2010) reported that acetate is a direct product of soluble carbohydrate fermentation, especially pectins, which is in agreement with the results observed in the current study. Other acids such as valerate and the branched chain acids isobutyrate and isovalerate are usually formed as a result of proteolysis (Macfarlane *et al.,* 1992; Williams *et al.,* 2001). In the present study, valeric acid was significantly reduced with the inclusion of ECP indicating that ECP could decrease the proportion or activity of proteolytic bacteria in the hindgut.

Fermentation of dietary fibre in the gastrointestinal tract results in decreased digesta pH (Jensen & Jørgensen, 1994) and increased production of organic acids, which, in turn, are capable of inhibiting some intestinal pathogens such as *E. coli* and *Salmonella spp.* (Montagne *et al.,* 2003; Ivarsson *et al.,* 2012). In our study, the inclusion of 150 g of ECP/kg in the diet reduced enterobacteria counts after 2 weeks of administration of the experimental diets, and this reduction increased with time. A reduction of enterobacteria counts has been also reported when fermented liquid feed is provided to growing pigs (van Winsen *et al.,* 2001; Canibe & Jensen, 2003). Dietary pH and VFA concentrations in the gut are considered the main factors affecting enterobacteria counts in these studies. Previous works with ECP from our group also showed a decrease in enterobacteria counts when feeding diets with 100 g of ECP/kg to finishing pigs (Cerisuelo *et al.,* 2010). Both, the high content of fermentable fibre and the diet characteristics derived from its fermentation (low pH and high VFA content) might explain this result. This effect on enterobacteria is of importance since it may decrease the infection pressure of enteropathogens in animals fed ECP. Lactic acid bacteria can tolerate better these circumstances. However, in the present study, lactobacilli counts were also reduced in treatments with 100 and 150 g of ECP/kg on week +5 leading to no changes in lactobacilli:enterobacteria ratio. Therefore, it seems that the inclusion of high ECP levels into pig diets might induce changes in the gut environment capable of acting against lactic bacteria.

Carcass quality

A crucial issue when evaluating the suitability of an unconventional feedstuff for animal nutrition is to know its effects on the final product. Diets that are rich in fermentable fibre are suggested to affect the quality of pork by means of reducing carcass yield or varying carcass fat content (Márquez & Ramos, 2007; Watanabe *et al*., 2010b; Crosswhite *et al*., 2013) due to its effects on gut fermentation. It has long been recognized that the weight and the volume of the whole gut, and of particular sections of it such as stomach, large intestine and caecum tend to increase in animals fed fibre-rich feeds (Kyriazakis & Emmans, 1995; Jørgensen *et al.,* 1996; Watanabe *et al*., 2010b; Ivarsson *et al.,* 2012) thus reducing carcass yield. In the present study, a reduction of two percentage units in carcass yield was observed in animals fed the diet including 150 g ECP/kg compared to animals fed the basal diet. Cerisuelo *et al.* (2010) also observed a reduction in carcass yield with the inclusion of 100 g ECP/kg in the diet. In the present study, a period of 20 h of fasting was practiced to the animals before slaughter. Thus, differences in gut weight could be related to the observed reduction in carcass yield, although this was not measured.

Fat depth at *gluteus medius* was quadratically affected by the ECP inclusion. Reductions of fat depth at *gluteus medius* (Cerisuelo *et al.,* 2010) or at backfat level (O'Sullivan *et al.,* 2003) have been observed with the inclusion of citrus by-products in diets. In the present study, pigs fed with 50 and 100 g of ECP/kg showed higher fat depth values compared to control and pigs fed the diet including 150 g of ECP/kg. Thus, it seems that the inclusion of ECP increases fat deposition at *gluteus medius* but when inclusion levels reach 150 g of ECP/kg, energy digestibility decreases and also fat deposition. The FA profile of the external subcutaneous fat layer was slighty affected by the addition of ECP in pig diets. However, the proportion of monounsaturated FA increased and that of polyunsaturated FA decreased in the internal layer in animals fed with 50 and 100 g of ECP/kg following a Q effect. Although an increase in the ratio of unsaturated to saturated FA in meat products is desirable due to health implications, the presence of a high percentage of polyunsaturated FA might impair meat quality because of reduced fat consistency (Wood & Enser, 1997) and, probably, also meat palatability. Thus, it seems that the inclusion of ECP at levels up to 100 g/kg diet might be beneficial in terms of carcass quality (high monounsaturated and low polyunsaturated FA), although the percentage of the different groups of FA observed in this experiment for all the treatments were maintained within the usual levels reported for pigs.

In conclusion, including up to 100 g of ECP/kg in finishing pig diets did not affect nutrient digestibility, carcass yield and subcutaneous fat FA profile. However, from the results obtained in the present study, higher inclusion levels (150 g of ECP/kg) are not recommended. The nutritional value of ECP in finishing pigs is low and its inclusion decreases faecal enterobacteria and lactobacilli counts.

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