RESEARCH ARTICLE

Effect of chestnuts level in the formulation of the commercial feed on carcass characteristics and meat quality of Celta pig breed

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Abstract

The effect of including chestnuts in the formulation of the feed on carcass characteristics and meat quality from 24 castrated males Celta pigs was studied. The inclusion of 15% of chestnut (CH15) improved ($p<0.01$) the carcass (118 *vs.* about 104 kg) and live weights (149 *vs.* 133-139 kg). Killing out percentage was also better for chestnuts groups than for control group. With regards the morphometric parameters, there were no statistically significant ($p>0.05$) differences except for the carcass length and ham length, for which the CH15 group proved to be the group with the longest sizes. The diet did not affect the physicochemical properties (colour parameters, water holding capacity and shear force) of *longissimus dorsi* muscle. The composition of some fatty acids of the *longissimus dorsi* muscle was affected by diet. The total saturated (35-38%) and total polyunsaturated fatty acids (8-10%) did not present differences. However, the increase of chestnut in the diet increased ($p<0.05$) the monounsaturated fatty acids in intramuscular fat (57% in CH25 *vs.* 53% in control and CH15). Within monounsaturated fatty acids, the C18:1n9 was the most influenced by the diet. The expression of the enzyme that synthesizes C18:1n9 depend on the composition of the diet. Therefore, the lower content of protein and the higher amounts of C18:1n9 and C18:2n6 in the chestnut could be explaining the greater content of C18:1n9 in muscle of chestnut-fed animals. The main conclusion is that including chestnuts in the diet would allow reduce production costs with no effect or even improving carcass measurements and meat quality.

Additional key words: carcass measurements; physicochemical parameters; diet, colour parameters; texture parameters; fatty acid profile.

Abbreviations used: C diet (concentrate feed diet); CH15 (concentrate feeding contained 15% of dried chestnuts); CH25 (concentrate feeding contained 25% of dried chestnuts); FA (fatty acids); h/H (hypocholesterolemic/hypercholesterolemic ratio); IMF (intramuscular fat); LD (*longissimus dorsi* muscle); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); SFA (saturated fatty acids); WHC (water holding capacity).

Introduction

In recent years, national and international institutions (European Union, FAO, UN, etc.) have increased their interest in maintaining world biodiversity and, to that end, they are supporting programs of Conservation and Recovery. In the specific case of the Celta pig, the number of animals is growing due to the help of Regional (Galician) and National (Spanish) programs, and to the new tendency, where many consumers, restau-

rants and industries have once again begun to demand and produce these traditional meat products.

Chestnut (*Castanea sativa* Mill.) is a Geographically Protected Identity (G.P.I.) (OJ, 2010) in the NW of Spain, since Galicia is the main production area in Spain. In order to increase the quality of the meat and meat products, the chestnuts could be used as ingredient in pig feeding. In Galicia, the extensive rearing of pigs, similar to *montanera* in Iberian pigs, is difficult due to the small size of the farms; thus, the feeding

with these products in large scale production can only be carried out by adding them to concentrated diets (Díaz *et al.,* 2009). Chestnuts are characterized by high moisture content (over 50%), high levels of starch (57 g/100 g dry matter), low protein level (5.8 g/100 g dry matter) and low fat content (3 g/100 g dry matter) (Pereira-Lorenzo *et al.,* 2006).

Currently, chestnuts are underutilized and this situation contrasts with the high current prices of commercial concentrates for animal feed. The use of chestnuts in the fattening feed, in an extensive management system, would allow the reduction of production costs and put in the market products of differentiated quality by a high added value and with healthier fat (Bermúdez *et al.,* 2012). In this regard, the smaller sized chestnuts and the by-products of this industry (pieces of chestnuts and chestnut meal) can be used in the elaboration of concentrated diets for pigs. So, the aim of this work was to study the effect of the level of chestnuts in the diet on carcass characteristics and meat quality of Celta pigs.

Material and methods

Experimental design and animal husbandry

The experiment was conducted with 24 barrows from Celta breed (Santiaguesa line). Pigs were reared, at the same time, in an extensive system in two experimental farms located in Lugo (Spain), with a forest of 960 m² (40 m² per animal) composed by *Eucalyptus* spp. and *Castanea sativa* trees. Camping tents for rest and pool for swimming are disposal in the area. The animals were fed a standard piglet diet for 3 months and then they were randomly divided into three groups (4 animals per group and farm × 3 groups × 2 farms). The animals were fed *ad libitum* in all groups. The first group was fed with a concentrate feeding (C diet) composed basically of cereals (barley, soy and wheat); the second and third groups were fed with concentrate feeding contained 15% and 25% of dried chestnuts (CH15 and CH25), respectively. The chemical composition of the chestnut was (on fresh matter basis): 51.9% dry matter, 4.2% crude protein, 3.3% ether extract, 2.0% crude fibre, 32.0% starch and 1.3% ash. The chemical and fatty acid (FA) composition of the diets are shown in Table 1. All diets provided 270-296 kcal/100 g of feed. The feed composition was determined according to AOAC (2000) procedures; total calorie estimates (kcal) were calculated on the basis of a 100 g portion using Atwater values for fat (9 kcal/g), protein (4 kcal/g), and carbohydrate (4 kcal/g) and FAs were determined as indicated in FA analyses section. Animals were slaughtered at the same day when they

Table 1. Chemical composition and fatty acid profile of the experimental feeds.

	Diet		
	C	**CH15**	**CH25**
Chemical composition (g/100 g of fresh matter)			
Moisture	10.50	11.20	11.00
Crude protein	15.90	14.20	13.30
Ether extract	4.40	4.20	4.00
Crude fibre	4.20	4.20	4.10
Ash	4.40	4.10	3.90
Starch	48.30	44.80	45.30
Energy (kcal/100 g)	296.5	273.8	270.4
Fatty acid profile (% of total fatty acids)			
C12:0	0.03	0.03	0.04
C14:0	0.89	0.82	0.75
C14:1	0.07	0.07	0.06
C15:0	0.12	0.11	0.11
C16:0	19.38	18.65	18.13
C16:1*n7*	1.44	1.35	1.25
C17:0	0.35	0.32	0.29
C17:1	0.19	0.18	0.17
C18:0	8.42	7.28	6.41
C18:1*n9*	29.85	30.48	30.72
C18:2*n6*	32.27	33.53	34.67
C20:0	0.26	0.27	0.27
C18:3*n3*	2.14	2.59	2.97
C20:3*n3*	0.03	0.03	0.03
C20:4*n6*	0.10	0.08	0.09
C20:5*n3*	0.13	0.13	0.13
C24:1*n3*	0.03	0.03	0.03
C22:5*n3*	0.03	0.02	0.02
C22:6*n3*	0.02	0.02	0.02

C: concentrate feeding (control); CH15: concentrate feeding contained 15% of dried chestnuts; CH25: concentrate feeding contained 25% of dried chestnuts

were 12 months old. Those from different batches were slaughter at the same age (363.2, 360.3 and 365.8 days for pigs of C, CH15 and CH25 respectively). The day before slaughter, all animals (from the two experimental farms) were weighed and transported to the abattoir trying to minimize the stress of the animals. The animals were slaughtered at 12 months by electrical stunning and exsanguinated at a commercial *abattoir* (Castro Riberas de Lea, Lugo, Spain).

Carcass measurements and sampling

The following data were recorded from all carcasses. At 45 min *post-mortem*, the pH was measured in the *longissimus dorsi* (LD) muscle between ninth

and tenth ribs using a digital pH-meter (Crison 507, Barcelona, Spain) equipped with a penetration probe. After 24 h *post-mortem* at 4 ºC, the cold weight of the carcass was measured using a slaughterhouse scale. The killing out percentage was calculated as the cold carcass weight expressed as a proportion of the slaughter weight. After, using a flexible tape measure, the following lengths and perimeters were measured on the hanging right half of the carcass: carcass length (from the middle of the cranial edge of the first rib to the ischiopubic symphysis), hand length (from the end of the olecranon to the distal point of the trotter) and maximum perimeter of the ham (in the area of maximum amplitude, near the base of the tail). Dorsal fat thickness was also measured in the middorsal line at the level of the mid point of the *Gluteus medius* muscle using a calibre. The carcass compactness index was calculated as cold carcass weight/carcass length.

Jointing was also carried out 24 h after slaughter. Head was first removed, by cutting between the occipital bone and atlas, and weighed; the jowls (right and left) were also removed and weighed. The following cuts were later obtained from each side, and weighed: loin, sirloin, ham, shoulder, backfat and bacon. Data taken from each carcass were the mean of the right and left cut. The carcass weight distribution was expressed as the weight of individual joints (right + left) (g) per 100 g of carcass and as percentage respect to carcass weight.

From all carcasses, a portion of the loin (LD muscle), between the fourth and tenth ribs, was taken for meat quality determinations. The loin samples were cut into four steaks, 2.5 cm thick. The first steak was used to determine colour parameters. The second and third steaks were used to determine water holding capacity (WHC) and texture parameters, respectively and the fourth was used for FA composition. The colour, WHC and the texture parameters were analysed on the day of collection while the fourth steak was minced, vacuum-packed and stored at -30 ºC for no longer than four weeks until FA analysis was carried out.

Meat quality

The following measures were carried out in pork samples at 24 h *post-mortem*. The pH was measured again following the method described previously. A portable colorimeter (Konica Minolta CR-300 Osaka, Japan) with the next settings machine (pulsed xenon arc lamp, angle of 0º viewing angle geometry and aperture size of 8 mm) was used to measure the meat colour in the CIELAB space (lightness, L*; redness, a*; yellowness, b* (CIE, 1978).

WHC was determined using the method of Grau & Hamm (1953) modified by Sierra (1973) and it was measured in three ways: press loss, drip loss and cooking loss.

To determine press loss, a 5 g sample of minced meat was placed between two disks of Whatman No. 1 filter paper (Filter Lab, Spain). After weighing the meat, a mass of 2.5 kg was applied for 5 min. The percentage of released water was calculated as:

$$\text{Press loss} = \frac{(\text{Initial fresh meat weight -Pressed weight })}{(\text{Initial fresh meat weight})} \times 100$$

To determine drip loss, a sample of intact meat (80–100 g and 1.5 cm thick) was weighed and placed on the top of a net, inside a closed container, in order to avoid evaporation into the environment. This container was placed in a chamber at 4 °C for 48 h and reweighed. The percentage drip loss was calculated as:

$$\text{Drip loss} = \frac{(\text{Initial fresh meat weight -Meat after 48 h weight})}{(\text{Initial fresh meat weight})} \times 100$$

To evaluate cooking loss, two 2.5 cm thick steaks were packed individually under vacuum (97%) (Tecnotrip model EV-15-1-D) and cooked in a water bath at 75 °C for 45 min (Selecta Tectron Bio, Barcelona, Spain). Samples were cooled at room temperature and cooking loss was calculated as follows:

$$\text{Cooking loss} = \frac{(\text{Initial fresh meat weight - Cooked weight})}{(\text{Initial fresh meat weight})} \times 100$$

To measure texture, two steaks were cooked as indicated for cooking loss. Steaks were cut into pieces (1×1×2.5 cm; height×width×length), parallel to the muscle fibre direction. They were completely sheared using a Warner Bratzler shear blade (3 mm thick) with a triangular cutting edge. A texture analyser (Stable Micro Systems TA-XT2, UK) was used and all samples were cut perpendicular to the muscle fibre direction at a crosshead speed of 2.5 mm/s. The average value for each sample was recorded from six to eight measurements.

Fatty acid analyses

Lipids were extracted from LD muscle following the Bligh & Dyer (1959) method. Methyl esters of the samples were produced according to the method of Morrison & Smith (1964). Fifty milligrams of the extracted lipids were esterified and the FA methyl esters (FAMEs) were stored at −80 °C until chromatographic analysis.

Separation and quantification of FAMEs was carried out using a gas chromatograph, Carlo Erba Instruments

MFC 500 auto/HRGC/MS (Milan, Italy) equipped with a flame ionization detector and using a Supelco DB-23 fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness, Supelco Inc., Bellafonte, PA, USA). Chromatographic conditions were as follows: initial oven temperature 170 °C (held for 2 min), first ramp at 3.5 °C/min to 210 °C and second ramp at 2.5 °C/min to 250 °C (held for 5 min). The injector and detector were maintained at 250 °C. Helium was used as carrier gas at a constant flow-rate of 2.0 mL/min. The split ratio was 1:50 and 1 μL of solution was injected. Nonadecanoic acid methyl ester (C19:0 ME) at 0.3 mg/mL was used as internal standard and added to the samples prior to fat extraction and methylation. Individual FAMEs were identified comparing their retention times with those of authenticated standards. Data regarding FAME composition were expressed as g/100 g of total identified FAMEs. The proportion of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids contents, PUFA/SFA, Σn-6/Σn-3, and the hypocholesterolemic/hypercholesterolemic ratio were calculated. The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated according to Fernández *et al.* (2007): h/H = [(sum of C18:1cn9, C18:1n7, C18:2n6, C18:3n6, C18:3n3, C20:3n6, C20:4n6, C20:5n3, C22:4n6, C22:5n3 and C22:6n3)/(sum of C14:0 and C16:0)].

Statistical analysis

A total of 24 pig carcasses and LD samples (four pigs for each batch × three batches × two farms) were analysed for different parameters. All statistical analysis was performed using IBM SPSS Statistics 19 software (IBM, Chicago, IL, USA). After verification of normal distribution and constant variance of data, the effect of different diets on carcass and colour parameters, WHC, Warner Bratzler test and FA profile was examined using a mixed-model ANOVA, where these parameters was set as dependent variables, the diets as fixed effect, and replicate (farms) as random effect. A Duncan´s test was performed to compare the mean values for processing time at a significance level of $p<0.05$. Correlations between variables were determined by correlation analyses using the Pearson's linear correlation coefficient.

Results and discussion

Carcass measurements

The effect of amount of chestnuts in the diet on live weight, carcass traits, morphology measurements and trimmed cuts are summarized in Table 2. Live weight (149 kg in CH15 group *vs.* 133 and 139 kg in C and CH25, respectively) and carcass weight (118 kg in CH15 group *vs.* 103 and 105 kg in C and CH25, respectively) were higher ($p<0.01$) in CH15 than in C and CH25 groups. This outcome is disagreement with those reported by Coutron-Gambotti *et al.* (1998), Pugliese *et al.* (2013) and Temperan *et al.* (2014) who concluded that the use of chestnuts in the finishing diet did not modify significantly these values. Killing out percentage was better for chestnuts groups (CH15 and CH25) than for control group, but no influence was observed for dorsal fat thickness (4.75 *vs.* 5.25 *vs.* 4.55 cm for C, CH15 and CH25 groups, respectively). This suggests that the use of chestnut in the diet does not affect carcass fatness or increase carcass quality without increasing the amount of fat, a fact that is sought in order to increase the lean/fat ratio. This finding is in agreement with data reported by Temperan *et al.* (2014) who did not find significant differences in dorsal fat thickness between pigs fed only with chestnuts (3 kg of chestnut/animal/day) during the three months before slaughter and those given commercial concentrate.

With regards the morphometric parameters, there were no significant differences except for the carcass length and ham length, for which the CH15 group proved to be the group with the longest carcass and ham, and the C group with the shortest. This fact could be related with animals from CH15 group also presenting the highest carcass size. These differences in the carcass length affected the weights of some trimmed. It was found from Pearson test showed (data not presented) that carcass length was positively correlated to head (r=0.530; $p<0.01$) and bacon (r=0.520; $p<0.01$) and negatively correlated to backfat depth (r=-0.556; $p<0.01$). We want to emphasize the correlations ($p<0.01$) between cold carcass weight and weight of the first quality meat cuts: r =0.862 with ham, r = 0.661 with loin and r = 0.664 with shoulder.

The proportions of ham and shoulder were higher ($p<0.05$) for CH15 group than for other ones, but no differences were detected for loin and sirloin. As a consequence, the combined proportion of primal cuts (ham, shoulder and loin) was higher for CH15 group (42.2 *vs.* 37.8 *vs.* 37.9% for CH15, C and CH25 groups, respectively). These findings are in disagreement with the data reported by Temperan *et al.* (2014) who concluded that the use of chestnuts in the finishing diet did not modify significantly the trimmed cuts weight.

Meat quality

The physicochemical properties of LD muscle from the pigs fed with the three different diets are shown in

Table 2. Effect of increasing dietary levels of chestnuts in carcass measures of Celta pigs

	Diet			SEM	p value
	C	CH15	CH25		
Live weight (kg)	139.3 ± 2.34^a	149.3 ± 2.13^b	133.3 ± 3.72^a	2.084	0.002
Carcass weight (kg)	103.6 ± 2.07^a	118.7 ± 2.47^b	105.5 ± 2.87^a	1.957	0.001
Killing out (%)	$74.3 \pm 0.55a$	$79.4 \pm 0.95b$	$79.2 \pm 1.00b$	0.682	<0.001
Carcass measurements (cm)					
Carcass length	103.2 ± 1.42^a	117.0 ± 1.29^b	112.4 ± 2.37^b	1.546	<0.001
Ham length	66.1 ± 1.80^a	71.7 ± 1.11^b	68.2 ± 1.01^{ab}	0.886	0.024
Ham perimeter	72.8 ± 1.10	74.9 ± 1.27	73.0 ± 1.14	0.683	0.395
Carcass compactness index	1.00 ± 0.01	1.01 ± 0.02	0.94 ± 0.03	0.010	0.088
Dorsal fat thickness	4.75 ± 0.26	5.25 ± 0.43	4.55 ± 0.31	0.195	0.341
Trimmed cuts weight (kg)					
Loin	4.00 ± 0.18	4.78 ± 0.30	4.21 ± 0.19	0.140	0.066
Sirloin	0.50 ± 0.03	0.53 ± 0.06	0.48 ± 0.03	0.021	0.667
Ham	23.41 ± 0.44^a	25.85 ± 0.68^b	24.10 ± 0.64^{ab}	0.391	0.025
Shoulder	9.36 ± 0.23^a	10.81 ± 0.44^b	9.30 ± 0.62^a	0.286	0.047
Backfat	7.18 ± 0.73^b	6.08 ± 0.84^{ab}	3.96 ± 0.76^a	0.508	0.026
Bacon	4.75 ± 0.09	5.07 ± 0.16	5.10 ± 0.18	0.095	0.372
Head	14.46 ± 0.38^a	16.95 ± 0.97^b	15.28 ± 0.32^{ab}	0.406	0.032
Loin + Ham + Shoulder	36.78 ± 0.62^a	41.45 ± 1.11^b	37.61 ± 1.39^a	0.728	0.014
Trimmed cuts (% respect to carcass)					
Loin	4.02 ± 0.19	4.80 ± 0.30	4.23 ± 0.19	0.142	0.066
Sirloin	0.50 ± 0.03	0.53 ± 0.06	0.48 ± 0.03	0.020	0.668
Ham	23.53 ± 0.44^a	25.97 ± 0.68^b	24.22 ± 0.64^{ab}	0.393	0.025
Shoulder	9.41 ± 0.23^a	10.87 ± 0.44^b	9.34 ± 0.62^a	0.291	0.047
Backfat	7.21 ± 0.74^b	6.11 ± 0.85^{ab}	3.98 ± 0.77^a	0.521	0.026
Bacon	9.54 ± 0.17	10.20 ± 0.32	10.27 ± 0.36	0.194	0.372
Head	14.53 ± 0.38^a	17.03 ± 0.97^b	15.36 ± 0.33^{ab}	0.413	0.032
Loin + Ham + Shoulder	37.85 ± 0.62^a	42.21 ± 1.12^b	37.99 ± 1.40^a	0.735	0.014

C: concentrate feeding (control); CH15: concentrate feeding contained 15% of dried chestnuts; CH25: concentrate feeding contained 25% of dried chestnuts; SEM = standard error of the mean; [a-b] Means in the same row with different letters differ significantly ($p<0.05$; Duncan test).

Table 3. On the other hand, the pH values measurement at 45 min or 24 h *post-mortem* did not show differences ($p<0.05$) among groups, although the increased amount of chestnuts in the diet led to a slight decrease in the ultimate pH values (5.69 *vs.* 5.62 *vs.* 5.55 for C, CH15 and CH25 groups, respectively). Intake of carbohydrates increases the muscle glycogen stores and it is well known that the glycogen content in the muscles affects both the rate and the extent of the decrease in pH *post mortem* (Henckel *et al.*, 2000). This result is agreement with those reported by Temperan *et al.* (2014) who observed slight lower pH values in Celta pig fed with chestnuts. However, Pugliese *et al.* (2013) noticed that pH values were significantly affected by the feeding system. In this case, the animals fed only with chestnuts during the month before slaughter presented the lowest values; however there was no difference between control animals and the animals fed only with chestnuts during the three months before slaughter. Therefore, there is no clear trend among chestnut inclusion in the diet and the pH value.

The colour parameters did not display differences among diets ($p>0.05$). These findings are in agreement with the data reported by Pugliese *et al.* (2007) and Temperan *et al.* (2014), who did not observe significant differences in colour parameters between pig fed with concentrate and chestnuts. In opposite, Pugliese *et al.* (2013) noticed an increase of the L*, a* and b* values in the *longissimus lumborum* muscle in pigs fattened with chestnuts during 1 or 3 months in comparison with pigs fattened without chestnuts. Authors attributed this effect to the tannin content of the chestnuts. As reported by Liu *et al.* (2009), in a study on rabbit reared indoor, the effects of chestnut tannins on meat colour could be related to the muscle iron content. Muscle iron content increased with increasing tannin level. In general, the

Table 3. Effect of increasing dietary levels of chestnuts in meat characteristics of Celta pigs

	Diet			SEM	*p* value
	C	CH15	CH25		
pH					
at 45 min *post-mortem*	6.14 ± 0.06	6.31 ± 0.07	6.23 ± 0.08	0.044	0.261
at 24 h *post-mortem*	5.69 ± 0.06	5.62 ± 0.08	5.55 ± 0.08	0.039	0.433
Colour parameters					
Lightness (L*)	47.43 ± 2.15	48.12 ± 1.38	49.36 ± 2.06	1.175	0.080
Redness (a*)	4.52 ± 0.50	6.23 ± 1.20	5.27 ± 0.87	0.520	0.423
Yellowness (b*)	7.18 ± 0.91	7.21 ± 0.36	8.21 ± 0.56	0.371	0.421
Water holding capacity indicators					
Pressing loss (%)	16.63 ± 1.44	19.30 ± 1.90	19.00 ± 0.44	0.813	0.354
Cooking loss (%)	17.94 ± 0.96	16.61 ± 1.39	18.16 ± 1.59	0.754	0.680
Drip loss (%)	1.49 ± 0.18	1.09 ± 0.16	1.34 ± 0.09	0.089	0.193
Warner Bratzler test					
Hardness (N)	63.36 ± 5.13	57.58 ± 3.85	59.40 ± 3.55	2.346	0.621

C: concentrate feeding (control); CH15: concentrate feeding contained 15% of dried chestnuts; CH25: concentrate feeding contained 25% of dried chestnuts; SEM = standard error of the mean.

rustic unimproved traditional breeds such as Celta, Chato Murciano, Iberian, etc. appear to have darker (lower L* values), redder (higher a* values) and more yellowish (higher b* values) meat compared to the improved breeds (Pietrain, Large White, Landrace, Yorkshire) and their crosses (Temperan *et al.,* 2014).

The WHC expressed as percentage of initial weight and measured by three different methods, was similar for all groups. In accordance with these results, Temperan *et al.* (2014) found that the inclusion of chestnuts in the finishing diet had no effect on it. In opposite, Pugliese *et al.* (2013) found significant differences on cooking and drip losses pigs fattened with chestnuts during 1 or 3 months in comparison with pigs fattened without chestnuts. These differences could be related to the higher fat content in pigs fattened with chestnuts, which contributes to the highest cooking losses because, as reported by Lawrie (1998), some of juice on cooking can represent non-aqueous fluid, since the high temperatures can melt fat and tend to destroy the structures retaining it. With regard to Warner Bratzler maximum shear force, statistical analysis did not show significant (*p*>0.05) differences among diets, as found in other studies where the effect of diet was studied (Pugliese *et al.*, 2013; Temperan *et al.,* 2014). Our mean values (about 60 N) were lower than those reported by Temperan *et al.* (2014) who found values above 100 N. These higher values of shear force could be due the older age at slaughtering (16 months) and the greater physical activity during rearing. However, Franco *et al.* (2014) reported values of Warner Bratzler ranging from 19.9 to 35.7 N and these lower Warner

Bratzler values could be related to the samples were frozen at -18 ºC for a week.

Fatty acid composition

Table 4 shows the effect of pigs fed on the FA composition of LD muscle. Statistical analysis did not show differences (*p*>0.05) in SFA content among groups, although the inclusion of chestnuts in the finishing diet seems to decrease the SFA content in the LD muscle (38.12%, 37.54% and 35.62% for C, CH15 and CH25 groups, respectively). This outcome is in agreement with the data reported by Temperan *et al.* (2014), who did not observe significant differences in SFA between pigs fed with concentrate and chestnuts. In opposite, Domínguez *et al.* (2015) found the lowest SFA values in fat from LD and *psoas major* muscles in Celta pigs fed only with chestnuts during the finishing diet. Pugliese *et al.* (2013) also noticed a significant decrease in SFA content in the *longissimus lumborum* muscle in pigs fattened with chestnuts during 1 or 3 months in comparison with pigs fattened without chestnuts. Differences in the composition of the diets of these studies could explain the differences in the content of SFA.

Within the SFA the predominant FA was palmitic acid (C16:0) which represented about 26% of total FAMEs followed by stearic acid (C18:0) (about 9%). The inclusion of the chestnuts in the diet only affected some minority FA [myristic acid (C14:0), pentanoic acid (C15:0), heptanoic acid (C17:0) and docosanoic acid (C22:0)]. The animals from CH25 presented the

Table 4. Effect of increasing dietary levels of chestnuts in fatty acid profile (expressed as % of total fatty acids) from intramuscular fat from *longissimus dorsi* muscle of Celta pigs

Fatty acids	Diet			SEM	*p* value
	C	CH15	CH25		
C12:0	0.24 ± 0.03	0.23 ± 0.04	0.22 ± 0.02	0.024	0.847
C14:0	0.02 ± 0.01[a]	0.10 ± 0.01[b]	0.03 ± 0.01[a]	0.012	<0.001
C14:1	2.39 ± 0.19	2.57 ± 0.26	2.23 ± 0.16	0.123	0.516
C15:0	0.08 ± 0.01[ab]	0.10 ± 0.01[b]	0.06 ± 0.01[a]	0.012	<0.001
C16:0	27.30 ± 0.24	26.31 ± 0.60	25.91 ± 0.72	0.350	0.279
C16:1*n7*	4.76 ± 0.18	5.69 ± 0.48	4.69 ± 0.34	0.232	0.133
C17:0	0.07 ± 0.01[a]	0.16 ± 0.03[b]	0.14 ± 0.02[b]	0.013	0.025
C17:1	0.30 ± 0.03	0.26 ± 0.02	0.23 ± 0.02	0.010	0.133
C18:0	9.98 ± 0.61	9.42 ± 0.68	8.04 ± 0.70	0.408	0.142
C18:1*n9*	45.10 ± 0.65[a]	45.40 ± 1.15[a]	49.93 ± 1.33[b]	0.810	0.010
C18:2*n6*	7.49 ± 0.28	8.49 ± 1.13	7.39 ± 1.34	0.529	0.686
C20:0	0.66 ± 0.06	0.76 ± 0.10	0.70 ± 0.06	0.036	0.478
C18:3*n3*	0.52 ± 0.05	0.60 ± 0.05	0.48 ± 0.08	0.042	0.599
C20:3*n3*	0.24 ± 0.03[c]	0.13 ± 0.01[b]	0.07 ± 0.01[a]	0.024	0.006
C20:4*n6*	0.05 ± 0.01[a]	0.37 ± 0.06[b]	0.30 ± 0.07[b]	0.045	<0.001
C20:5*n3*	0.30 ± 0.05[a]	0.39 ± 0.05[b]	0.29 ± 0.04[a]	0.042	0.020
C22:0	0.34 ± 0.05[c]	0.10 ± 0.01[b]	0.05 ± 0.01[a]	0.044	<0.001
C22:4*n3*	0.09 ± 0.01	0.12 ± 0.02	0.10 ± 0.02	0.009	0.528
C22:6*n3*	0.12 ± 0.01	0.13 ± 0.01	0.09 ± 0.03	0.012	0.302
C24:1*n9*	0.10 ± 0.02[a]	0.13 ± 0.02[a]	0.20 ± 0.02[b]	0.010	0.021
ΣSFA	38.12 ± 0.87	37.54 ± 0.73	35.62 ± 1.32	0.632	0.224
ΣMUFA	53.03 ± 0.59[a]	53.70 ± 1.79[a]	57.50 ± 1.32[b]	0.833	0.039
ΣPUFA	8.84 ± 0.41	10.26 ± 1.22	9.69 ± 1.94	0.644	0.663
PUFA/SFA	0.22 ± 0.01	0.28 ± 0.02	0.24 ± 0.04	0.021	0.430
Σ*n3*	0.99 ± 0.07	1.02 ± 0.10	0.94 ± 0.22	0.091	0.915
Σ*n6*	7.59 ± 0.26	8.85 ± 1.13	7.19 ± 1.24	0.549	0.481
Σ*n6*/Σ*n3*	8.23 ± 0.43	8.59 ± 0.52	8.28 ± 0.75	0.323	0.892
h/H	2.02 ± 0.09	2.09 ± 0.07	2.20 ± 0.11	0.052	0.141

C: concentrate feeding (control); CH15: concentrate feeding contained 15% of dried chestnuts; CH25: concentrate feeding contained 25% of dried chestnuts; SEM = standard error of the mean; [a-b] Means in the same row with different letters differ significantly ($p<0.05$; Duncan test); PUFA = Σ (C18:2*n6* + C18:3*n3* + C20:3*n3* + C20:4*n6* + C20:5*n3* + C22:4*n3* + C22:6*n3*); MUFA = Σ (C14:1 + C16:1*n7* + C17:1 + C18:1*n9* + C24:1*n9*); SFA = Σ (C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0); Σ*n-6* = Σ (C18:2*n6* + C20:4*n6*); Σ*n-3* = Σ (C18:3*n3* + C20:3*n3* + C20:5*n3* + C22:4*n3* + C22:6*n3*); h/H: The hypocholesterolemic/hypercholesterolemic ratio = [(sum of C18:1*n9*, C18:1*n7*, C18:2*n6*, C18:3*n6*, C18:3*n3*, C20:3*n6*, C20:4*n6*, C20:5*n3*, C22:4*n6*, C22:5*n3* and C22:6*n3*)/(sum of C14:0 and C16:0)] (Fernández *et al.*, 2007)

lowest values of C16:0 (25.91% *vs*. 26.31% *vs*. 27.30% for CH25, CH15 and C groups, respectively) and C18:0 (8.04% *vs*. 9.42% *vs*. 9.98% for CH25, CH15 and C groups, respectively) although these differences were not significant. These results agree with other authors (Temperan *et al.*, 2014; Domínguez *et al.,* 2015). According to Kloareg *et al.* (2005), the *de novo* synthesis of the main five non-essential FA (C14:0, C16:0, C16:1*n7*, C18:0 and C18:1*n9*) represented 98% of the total *de novo* synthesized FA and 92% of the total deposited FA. The carbohydrates serve as substrate in the synthesis of fat, producing palmitic acid. Therefore,

the highest content of starch in C diet (48.3 *vs*. 44.8 *vs*. 45.3 g/100 g for C, CH15 and CH25 diets, respectively; see Table 1) could partly explain the greater ($p>0.05$) amount of C16:0 in animals fed with C diet than animals fed with the other two diets.

Regarding to MUFA content, the increase of chestnut in the finishing diet seems to increase ($p<0.05$) their proportion in intramuscular fat (IMF). Animals fed with CH25 presented higher values of MUFA (57.50%) than animals fed with CH15 (53.70%) and C diets (53.03%). Within MUFA, only C18:1n9 ($p<0.01$) and C24:1n9 ($p<0.05$) showed differences among groups. The

amount of C18:1n9 was higher ($p<0.05$) for CH25 group (49.93%) than for other ones (45.40 and 45.10% for CH15 and C groups, respectively). These results agree with those described by Bermúdez et al. (2012) in hams and by Domínguez et al. (2015) in psoas major muscle. These authors found that the increase of chestnut amount in the finishing diet strongly increased the C18:1n9 percentage in pork meat. In contrast, Temperan et al. (2014) did not find differences in MUFA or C18:1n9 amounts in LD and neither in semimembranosus muscles between animals feed with chestnuts, mixed diet and compound feed. The high MUFA percentage observed in meat from CH25 group indicates its suitability for healthier diets, due to that human diets rich in MUFA (and PUFA) decrease cholesterol levels in blood and are related to a low incidence of cardiovascular diseases (Alexander, 1998; Kris-Etherton, 1999).

According to Domínguez et al. (2015), the highest content of C18:1n9 in IMF from chestnut-fed pigs is related to the fact that this diet had higher contents of this FA and C18:2n6 and lower contents of protein and retinol compared to the compound feed. Bermúdez et al. (2012) also found positive correlation between MUFA meat content and concentrations of C18:1n9 in diets.

It has been suggested that fat deposition in different depots might be regulated by different mechanisms (Gondret et al., 2008) and stearoyl-CoA desaturase plays the key role in this process (Doran et al., 2006). It is well known that the expression of stearoyl-CoA desaturase in LD muscle increase with low protein diets (Doran et al., 2006; Wood et al., 2008; Cánovas et al., 2009). This fact agrees with those reported by Domínguez et al., (2015), who found a negative relationship between C18:1n9 content in IMF deposition and the dietary protein. Therefore, knowing that there is a linear relationship between the expression of this enzyme and the amount of C18:1n9 in muscle, the higher percentage of this FA in CH25 group could be related with the fact that CH25 diet had the lowest content of protein and the highest content of C18:1n9.

The PUFA content did not show differences among groups ($p>0.05$). This fact agrees with the results described by Temperan et al. (2014) and Domínguez et al. (2015), who did not find differences in PUFA content between pigs fed with chestnut and commercial feed. In contrast, Coutron-Gambotti et al. (1998) reported higher contents in PUFA in the biceps femoris muscle in pigs fed with chestnuts. In the present study, only three minority PUFA presented differences according to diet. Animals fed with CH15 and CH25 diets had the highest amounts of arachidonic acid (C20:4n6) (0.30% vs. 0.37% vs. 0.05% in CH25, CH15 and C

group, respectively) and the lowest amounts of eicosatrienoic acid (C20:3n3) (0.07% vs. 0.13% vs. 0.24% in CH25, CH15 and C group, respectively). The CH15 group showed the highest content of eicosapentaenoic acid (C20:5n3) (0.39% vs. 0.29% vs. 0.30% in CH15, CH25 and C group, respectively). Bermúdez et al. (2012) also found that an increase of chestnut in the finishing diet increased the amount of C20:4n6 in hams. This FA proceeds from processes of elongation and desaturation of C18:2n6 (Enser et al., 2000; Pérez-Palacios et al., 2009) or it can also be incorporated directly by diet. Our results suggest the importance of the synthesis, since an increased amount of C18:2n6 in the diet (including chestnuts) increases levels of C20:4n6 in meat.

The C18:2n6 and C18:3n3 contents in the porcine tissues are directly related to the contents of these two FA in the diet, because they cannot be synthesised in the tissues. Therefore, in our study a higher incorporation of C18:2n6 and C18:3n3 in animal tissues with an increase of chestnut in the diet would be expected. However, although CH15 and CH25 diets had higher amounts of these FA than C diet (see Table 1), the contents of C18:2n6 and C18:3n3 in IMF did not show differences among groups. This result agrees with those previously described by Temperan et al. (2014) and Domínguez et al. (2015), who did not find differences in these FA as chestnut increased in the diet. In contrast, Bermúdez et al. (2012) found an increase of C18:3n3 and a decrease of C18:2n6 in animals fed only with chestnut in comparison with animals fed with commercial feed.

The Δ6-desaturase is one of the enzymes that catalyse the conversion of the essential FA into long-chain PUFA in animal tissues (Domínguez et al., 2015). However, it has been recently reported that C18:1n9 itself can reduce Δ6-desaturase activity (Portolesi et al., 2008), so C18:2n6 would be replaced by other energy sources such as C18:1n9. This could also be related to the fact that C18:2n6 did not show differences among groups. In addition, Warnants et al. (1996) reported a lower efficiency in the incorporation of dietary FA in the muscle than in the subcutaneous fat. On the other hand, Leszczynski et al. (1992) and Domínguez et al. (2015) also observed that LD muscle is less influenced by diet and is less sensitive to the incorporation of dietary PUFA than dorsal fat.

The PUFA/SFA ratio, the PUFA n6/n3 ratio and the h/H ratio were calculated. There were no significant differences of these ratios among animals fed with the three diets. In relation to PUFA/SFA, a value above 0.4 is recommended for healthy foods and diets (UK Department of Health, 1994) although, the high proportion of PUFA in itself is not necessarily healthy if it is not balanced in relation to the n6/n3 ratio, which should

not exceed 4 (Simopoulos & Cleland, 2003; Simopoulos, 2004). Excessive amounts of n6 PUFA and very high n6/n3 PUFA ratios promote several kinds of pathogenesis, including cardiovascular disease, cancer and inflammatory and autoimmune diseases, whereas increased levels of n3 PUFA (and low n6/n3 PUFA ratios) exert suppressive effects (Simopoulos, 2004). A better approach should be the use of h/H ratio, based on the functional effects of FA on cholesterol metabolism (Santos-Silva *et al.,* 2002). In the present work, meat had PUFA/SFA ratio lower values than recommended (0.22–0.28), while the n6/n3 ratio was higher (about 8.30) than the values recommended (4). Finally, the values of h/H ratio (2.02-2.20) were similar to those described by Bermúdez *et al.* (2012) (2.21-2.46) and by Domínguez & Lorenzo (2014) (1.78-2.14). Although the h/H values did not show differences, increasing the amount of chestnut in the diet, also increase h/H value (2.20 *vs*. 2.09 *vs*. 2.02 in CH25, CH15 and C, respectively. As a result, from a healthy point of view, the h/H ratio of the meat from the CH25 diet was more favourable than from the C group.

As a general conclusion, the inclusion of chestnuts in the formulation of feed increased the live and carcass weights and killing out percentage. Animals from CH15 group showed the highest proportions of ham and shoulder. Therefore, the use of 15% of chestnuts in the diet led to Celta pigs with high weights and percentages of the main cuts and slight higher levels of carcass fat. In contrast, the inclusion of chestnut in diet had no significant effect on the meat quality (colour, WHC and shear force). Regarding FA composition, the use of chestnut in the diet seems to decrease the SFA (although this effect was not statistically significant in the present study) content and increase the MUFA content in the pig muscle. Therefore, the inclusion of chestnuts in the pig diet did not affect or even improved some carcass measurements and meat quality traits and also would allow reduce production costs.

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