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Effect of the use of chestnuts in the finishing diet on fatty acid profile in different tissues of the Celta pig breed

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Abstract. The effect of the use of chestnuts in the finishing diet on the fatty acid profiles of different tissues (muscles and liver) of the Celta pig (an autochthonous breed from the NW of Spain) was studied. Thirty six pigs were separated in three different groups according to the type of feeding during the finish-fattening period (three months): commercial compound feed, mixture of the chestnuts and commercial compound feed, and chestnuts. Fatty acid composition of neutral (NL) and polar lipids (PL) in the intramuscular fat of *Longissimus dorsi* (LD) and *Psoas maior* and *minor* (PMM) muscles and in the hepatic fat were analysed. The predominantly oxidative PMM muscle had higher polyunsaturated and lower monounsaturated fatty acids contents than LD both in NL and PL. The oleic acid (C18:1 n-9) content in neutral lipids and linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acid contents in polar lipids showed significant higher values in the LD and PMM muscles from pigs fed only with chestnuts. Also, the inclusion of chestnuts in the diet significantly affected the palmitic (C16:0), stearic (C18:0) and oleic (C18:1 n-9) acid contents in both lipid classes in liver; the arachidonic (C20:4 n-6) and linolenic (C18:3 n-3) acids contents were also affected in NL and PL, respectively.

Keywords. Celta pig breed – Lipids – Finishing diet – Fatty acid – Chestnuts.

Effet de l'utilisation de la châtaigne dans l'alimentation de finition sur le profil en acides gras dans les différents tissus du porc de race Celta

Résumé. *L'effet de l'utilisation de la châtaigne dans l'alimentation de finition sur les profils en acides gras de différents tissus (muscles et foie) de porcs Celta (une race autochtone du nord-ouest de l'Espagne) a été étudié. Trente-six porcs ont été séparés en trois groupes différents selon le type d'alimentation au cours de la période de finition-engraissement (trois mois): aliment composé commercial, mélange de châtaignes et d'aliment composé commercial, et châtaignes. La composition en acides gras des lipides neutres (LN) et polaires (LP) dans la graisse intramusculaire des muscles* Longissimus dorsi *(LD) et* Psoas maior *et* minor *(PMM) et dans la graisse hépatique a été analysée. Le muscle PMM, avec un métabolisme principalement oxydatif, a montré des teneurs plus élevées en acides gras polyinsaturés et plus basses en acides gras monoinsaturés que le muscle LD, tant pour les LN que pour les LP. La teneur en acide oléique (C18:1 n-9) dans les lipides neutres et la teneur en acides linoléique (C18:2 n-6) et linolénique (C18:3 n-3) dans les lipides polaires montrent des valeurs plus élevées, avec une différence significative, dans les muscles LD et PMM des porcs nourris seulement avec des châtaignes. En outre, l'inclusion des châtaignes dans l'alimentation a affecté d'une façon significative les teneurs en acides palmitique (C16:0), stéarique (C18:0) et oléique (C18:1 n-9) dans les deux classes de lipides dans le foie; les teneurs en acides arachidonique (C20:4 n-6) et linolénique (C18 3 n-3) ont également été affectées dans les LN et LP, respectivement.*

Mots-clés. *Race porcine Celta – Lipides – Alimentation de finition – Acides gras – Châtaignes.*

I – Introduction

Celta pig is an autochthonous swine breed from the NW of Spain, characterized by its rusticity and its adaptation to the environment. It is catalogued as Special Protection pig breed in danger of extinction (BOE 21/11/1997, R.D. 1682/1997) because was substituted with the arrival of the

commercial crossbreds with higher productive capacity during the 1990s. At the present time, this breed is recovering and the carcasses are used in the production of dry meat products which have a high value on the market.

The muscles differ in the amount and fatty acid composition of the main lipid fractions, neutral lipids and phospholipids. The influence of diet on the fatty acid composition of animal tissues, particularly muscle and adipose tissue, has been the subject of much investigation (Wood *et al*., 2008). An efficient way of influencing the fatty acid composition in pork is by feeding sources with varying fatty acid composition. The desired fatty acid composition in meat products should give appropriate pork quality: shelf life, flavour and high nutritional value (Hallenstvedt *et al*., 2010).

The use of the chestnuts (NW region is the main area of production in Spain) in the feeding of the Celta pig breed, in a extensive management system, would allow reducing the production costs and puting in the market quality products, differentiated, with a high added value and with healthier fat. In previous studies (Franco *et al*., 2006; Martínez *et al*., 2007) were investigated the fatty acid profile of the total, neutral and polar lipids in different deposits of Celta pigs fed with traditional diet.

The objetive of this study was to assess the effect of the inclusion of chestnuts in the finishing diet on the fatty acid composition of neutral and polar lipids in intramuscular and hepatic fat of Celta pigs.

II – Materials and methods

1. Pigs, samples and diets

In order to carry out this study, 36 castrated Celta pigs (males and females) were fed in three different groups: A) Fed during all their life (16 months) with commercial compound feed, B) Fed with commercial compound feed the first 12 months and with a mixed (commercial compound feed/chestnuts) diet in the last four months before slaughtering and C) Fed with commercial compound feed the first 12 months, with a mixed (commercial compound feed/chestnuts) diet the 13th month, and receiving only a chestnut diet in the last three months before slaughtering. After slaughtering, and after 24 hours of refrigeration, were obtained in each carcass samples from intramuscular fat – *Longissimus dorsi* (LD) and *Psoas maior* and *minor* (PMM) muscles – and hepatic fat.

Chestnuts and commercial compound feed were sampled and was determined the chemical composition according to the Association of Official Analytical Chemist (1990) (Table 1). The fatty acids profile was determined as shown in the following paragraph.

2. Analytical methods

The fat of the samples was extracted following the procedure described by Folch *et al*. (1957). The neutral and polar lipids from muscles and liver samples were obtained according to the procedure developed by Kaluzny *et al*. (1985). Fat extracts were methylated and fatty acid profiles of the both lipids were determined using the procedure described by Franco *et al*. (2006). Fatty acid methyl esters were analysed by Gas Chromatography using a Thermo Finnigan Trace GC (Thermo Finnigan Trace GC (Thermo Finnigan, Austin, TX, USA) The separation of the different fatty acids was carried out in an Innowax column: 30 m; 25mm ID; 0.25 mm film thickness (Agilent Technologies, Palo Alto, CA, USA). The temperature of the detector was 250ºC and that of the injector 230ºC. The gasses used were air (350 mL/min), hydrogen (335 mL/min) and helium (carrier gas) (30 mL/min). Results are expressed as percentages of the total fatty acid composition. All analyses were carried out in duplicate.

Table 1. Chemical composition (expressed as g/100 g) and fatty acids of chestnuts and commercial compound feed

	Chestnut	Compound feed
Dry matter	51.9	89.5
Crude protein	4.2	15.3
Eter extract	3.3	4.9
Crude fiber	2	4.6
Starch	32	39.7
Fatty acids (g/100 g total fatty acids)		
C12:0	0.03±0.00	0.16±0.01
C14:0	0.15±0.00	1.28±0.06
C14:1	N.D.	0.12±0.01
C15:0	0.10±0.00	0.16±0.01
C15:1	N.D.	0.04±0.00
C16:0	15.88±0.04	20.83±0.63
C16:1	0.48±0.00	1.75±0.06
C17:0	0.13±0.00	0.42±0.00
C17:1	0.06±0.00	0.21±0.00
C18:0	1.41±0.02	9.00±0.32
C18:1 *n-9*	26.05±0.07	24.85±0.30
C18:2*n*-6	40.47±0.32	24.04±0.00
C18:3*n*-3	5.94±0.09	2.58±0.04
C20:0	0.28±0.00	0.15±0.00
C18:3*n*-6	0.41±0.00	0.41±0.01
C20:2*n*-6	0.22±0.02	0.03±0.00
C20:3*n*-6	0.17±0.06	0.01±0.00
C20:4*n*-6	2.57±0.02	7.03±0.11
C24:0	5.65±0.14	6.93±0.01

3. Statistical analysis

Data was subjected to a two-way analysis of variance (ANOVA) using the General Linear Model procedure of the computer programme Statistica© 5.1 for Windows (Statsoft Inc., Tulsa, OK, USA) to determine the overall effect of muscle (LD *vs* PMM) and diet (commercial compound feed, mixture of the chestnuts and commercial compound feed, and chestnuts).

III – Results and discussion

Fatty acid compositions of NL and PL from LD and PMM muscles are shown in Table 1. In NL, LD muscle showed higher levels ($P<0.05$) of UFA and MUFA and lower ($P<0.05$) of PUFA than PMM. These differences are a direct consequence of higher amounts of palmitoleic (C16:1) and oleic (C18:1 n-9) acids in LD muscle, and higher amounts of palmitic (C16:0), linoleic (C18:2 n-3), C20:2 n-6, arachidonic (C20:4 n-6) and C20:3 n-6 acids in PMM muscle.

The total amount of MUFA and PUFA in PL was also affected by the type of muscle, PMM muscle showing higher levels ($P<0.05$) of PUFA and lower ($P<0.05$) of MUFA than in the NL. These differences are a direct result of higher proportions of linoleic (C18:2 n-3), linolenic

(C18:3 n-3), arachidonic (C20:4 n-6) and C20:3 n-6 acids in PMM muscle and higher proportions of oleic (C18:1 n-9) acid in LD muscle.

PMM is a predominantly oxidative muscle, showing in both lipids class higher levels of PUFA than LD, which has been described as a predominantly glycolytic muscle in the scientific literature (Muriel *et al*., 2002). With regards to other publications studying the effect of the metabolic type muscle on the fatty acid composition of NL and PL is some controversy (Andrés *et al*., 2001; Hernández *et al*., 1998; Alasnier *et al*., 1996; Leseigneur-Meynier and Gandemer, 1991). Leseigneur-Meynier and Gandemer (1991) have found a similar tendency: oxidative muscles have higher PUFA contents in PL and NL.

The fatty acid composition of NL and PL of liver are shown in Table 2. In NL, liver had a higher percentage of PUFA than LD and PMM muscles. These differences are a direct consequence of higher amounts of linoleic (C18:2 n-3) and arachidonic (C20:4 n-6) acids in liver. The profile of fatty acid that we observed in the liver coincides with that found in a previous study by Martínez *et al*. (2007).

Table 2. Fatty acid composition, % and standard error of the mean (SEM), of neutral and polar lipids in LD and PMM muscles

	Neutral lipids (NL)				Polar lipids (PL)			
	LD		*PMM*		*LD*		*PMM*	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM.**	**Mean**	**SEM**
C12:0	0.05	0.006	0.05**	0.006	0.02_a	0.005	0.01_b	0.002
C14:0	1.37	0.016	1.39	0.021	2.45_a	0.138	1.87_b	0.149
C14:1	0.03_a	0.002	0.04_b	0.003	1.03	0.174	0.71	0.136
C16:0	24.97_a	0.139	26.03_b	0.160	28.20_a	0.279	26.29_b	0.329
C16:1	3.62_a	0.091	$3.01*_b$	0.089	1.16_a	0.038	0.84_b	0.035
C17:0	0.20_a	0.008	0.31_b	0.013	0.23*	0.014	0.25	0.015
C17:1	0.19_a	0.006	0.22_b	0.009	1.16	0.113	1.31	0.142
C18:0	$13.37*_a$	0.289	14.35_b	0.256	$5.13**_a$	0.185	6.93_b	0.289
C18:1n-9	$46.21*_a$	0.407	$40.76**_b$	0.423	14.30_a	0.366	10.97_b	0.278
C18:2n-6	7.75_a	0.201	11.26_b	0.316	$33.07*_a$	0.383	$35.73*_b$	0.430
C18:3n-3	0.44_a	0.017	0.67_b	0.020	$0.50***_a$	0.020	$0.61***_b$	0.025
C20:0	0.20_a	0.005	0.17_b	0.005	0.06	0.009	0.06	0.008
C18:3n-6	0.90_a	0.022	0.79_b	0.018	0.25	0.017	0.23**	0.010
C20:2n-6	0.37_a	0.016	0.47_b	0.021	0.47***	0.033	0.53***	0.039
C20:4n-6	0.18_a	0.009	0.27_b	0.012	$10.34**_a$	0.324	$12.01**_b$	0.416
C22:0	n.d.		n.d.		0.18	0.033	0.17	0.035
C20:3n-6	0.08_a	0.006	$0.11*_b$	0.011	0.67*	0.077	0.59***	0.060
C24:0	$0.06*_a$	0.004	0.08_b	0.004	0.67*	0.055	0.76	0.059
SFA	40.22_a	0.352	42.40_b	0.361	37.03	0.350	36.48	0.353
UFA	59.78_a	0.352	57.60_b	0.361	62.97*	0.350	63.52*	0.353
MUFA	$50.06*_a$	0.447	$44.03**_b$	0.465	17.66_a	0.364	13.83_b	0.300
PUFA	9.72_a	0.228	13.57_b	0.348	$45.31*_a$	0.530	$49.69*_b$	0.395

LD: *Longissimus dorsi*; *PMM*: *Psoas maior* and *minor.* SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids.

[a,b]Means within the same row and lipid class not followed by the same number differ significantly ($P<0.05$). Significantly diferent values as influenced by diet *($P<0.05$); **($P<0.01$); ***($P<0.001$).

The fattening diet clearly affects the fatty acid composition of NL and PL in both muscles and liver. (Table 3). The oleic acid (C18:1 n-9) content in NL and linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acid contents in PL showed significant higher values in the LD and PMM muscles from pigs fed only with chestnuts. In these muscles, also the linolenic (C18:3 n-3) and arachidonic (C20:4 n-6) acids content in the PL was affected by diet. No significant differences were observed in SFA composition associated to the type of finishing diet.

Table 3. Fatty acid composition, % and standard error of the mean (SEM), of neutral and polar lipids in liver

	Neutral lipids (NL)		Polar lipids (PL)	
	Mean	**SEM**	**Mean**	**SEM**
C12:0	n.d.		n.d.	
C14:0	0.40	0.031	0.40	0.029
C14:1	0.03	0.006	0.09	0.006
C16:0	15.06	0.412	20.44	0.419
C16:1	0.95*	0.057	0.96	0.047
C17:0	0.45	0.031	0.60	0.045
C17:1	0.18	0.009	0.19	0.013
C18:0	18.55***	0.411	28.60***	0.803
C18:1n-9	25.89***	0.529	13.85***	0.652
C18:2n-6	17.99	0.332	17.62	0.363
C18:3n-3	0.80	0.036	0.57*	0.034
C20:0	0.04	0.003	0.04	0.010
C18:3n-6	0.33	0.014	0.18	0.022
C20:2n-6	0.42	0.021	0.58	0.033
C20:4n-6	16.59***	0.655	13.93	0.407
C20:3n-6	0.72	0.026	0.46	0.022
C24:0	1.58	0.106	1.41	0.117
SFA	36.09**	0.492	51.58***	0.693
UFA	63.91**	0.492	48.42***	0.693
MUFA	27.06***	0.569	15.10***	0.644
PUFA	36.85***	0.796	33.33	0.615

SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids.
Significantly diferent values as influenced by diet *($P<0.05$); **($P<0.01$); ***($P<0.001$).

It has been proposed that liver fatty acid composition may be a good indicator of feeding regime received by the pigs during the last fattening days (Ruiz *et al*., 1998). In this experiment was observed that inclusion of chestnuts in the diet significantly affected the palmitic (C16:0), stearic (C18:0) and oleic (C18:1 n-9) acid contents in both lipid classes in liver; the arachidonic (C20:4 n-6) and linolenic (C18:3 n-3) acid contents were also affected in NL and PL, respectively.

The factors associated with feeding that modify the composition in fatty acids of the tissues of the pig are: the amount of fat, proteins and carbohydrates present in the diet, the composition in fatty acids of the meal and the duration of the fattening period (Cava and Andrés, 2001).

Oleic acid (C18:1 n-9), is the most abundant fatty acid in NL of fat intramuscular and liver. The results show that the chestnuts inclusion increased the deposition of oleic acid (C18:1 n-9) in NL of both muscles and in NL and PL of liver. Doran *et al*. (2006) and Teye *et al*. (2006) showed

that low protein diets increased the expression of stearoyl Co-A desaturase in *Longissimus dorsi* muscle and that there is a linear relationship between the expression of stearoyl Co-A desaturase and the amount of oleic acid (C18:1 n-9) in muscle. The protein content is lower in chestnuts than in the commercial feed compound, which would explain in part the high deposition of oleic acid (C18:1 n-9) in LN, fundamentally.

The linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids content of porcine fat is related to the linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids of the diet. The chestnuts have higher levels of linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids that commercial compound (Table 1). We found increased level of linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids in PL of LD and PMM muscles. Enser *et al*. (2000) showed that an increased dietary level of linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids results in an increased level of these fatty acids in both neutral and polar lipids of intramuscular fat. In other hand, the triglyceride content has a low fatty acid turnover rate and metabolic activity, average triglyceride life has been estimated to be over 180 days (Cunningham, 1968), a longer time than fattening phase of Celta pigs. Consequently, the concentration of the linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids does not accurately reflect the ingestion of chestnuts during the finish-fattening phase in NL.

The biosynthesis of arachidonic acid (C20:4 n-6) involves the desaturation and elongation of the dietary linoleic acid (C18:2 n-6) (Valette *et al*., 1991; Pérez-Palacios *et al*., 2009). The chestnuts inclusion in diet significantly affected the content of arachidonic acid (C20:4 n-6) on the PL of both muscles ($P<0.01$) and in the NL of liver ($P<0.001$).

The C20:2 n-6 and C20:3 n-6 acids were also affected on the PL of both muscles by the inclusion of chestnuts in the diet. These fatty acids derived from dietary linolenic acid (C18:3 n-3) and are deposited in muscle phospholipids but not in muscle neutral lipid (Enser *et al*., 2000), as shown in the present study.

IV – Conclusions

Metabolic type of muscle influences fatty acid composition of NL and PL in swine, those muscles with a predominantly oxidative metabolism showing higher amounts of PUFA in both lipid classes. On the other hand, we can observe a high response in fatty acid composition of in intramuscular and hepatic fat, as a result of chestnuts inclusion in the diet during the finish-fattening period.

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