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Acorn chemical composition depending on shedding date and *Quercus* species

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Abstract. Acorns from *Quercus* species are the main feed resource of free range Alentejano pigs during their fattening period. The animals have access to the acorns as they shed, along the "Montanheira" season. It is well known that chemical composition of acorns vary with species, botanical varieties, harvest year and shedding date. A better knowledge of the chemical composition of acorns along the "Montanheira" may result in a more rational utilization of acorns by pigs and therefore a better management of the "Montanheira". The aim of this study was to evaluate the chemical composition of acorns from Q. *rotundifolia* and *Q. suber*, from October to December. Acorn samples from eight trees were collected from the ground every two weeks (6 collection dates). Acorns were separated into hull and kernel, freeze dried, ground and analyzed for dry matter, ashes, protein, fat, NDF, ADF, starch and tannins. There was an effect of species on the chemical composition, specially for protein content which was about twice as high in the seed of *Q. suber* (83,2 g/kgDM) than in that of *Q. rotundifolia* (39,8 g/kgDM). Date of shedding affected mostly tannin content of acorns, which decreases along maturation process, as it was observed by previous authors.

Keywords. Acorn – Chemical composition – *Quercus rotundifolia* – *Quercus suber* – Shedding date.

Composition chimique des glands en fonction de la date de récolte et l'espèce (*Quercus rotundifolia vs Quercus suber*)

Résumé. *Le gland de* Quercus rotundifolia *et* Quercus suber *est la principale source de nourriture dans la finition des porcs de l'Alentejo en régime extensif. Les animaux ont accès au gland quand il tombe des arbres au cours de la période de «montanheira». Il est bien connu que la composition chimique des glands varie selon les espèces, les variétés botaniques, l'année de récolte et la date de récolte. Une connaissance plus détaillée de la composition chimique au cours de la «montanheira» peut entraîner une utilisation plus rationnelle des glands et, par conséquent, une meilleure gestion des «montanheira». Le but de cette étude était d'évaluer la composition chimique des glands* (Q. rotundifolia *et* Q. suber*), entre le début octobre et la fin décembre. Des échantillons de glands de huit arbres ont été prélevés sur le sol tous les 15 jours (six dates de récolte), séparés en coquille et graine, lyophilisés, broyés et analysés pour la matière sèche, cendres, protéines, graisse, NDF, ADF, amidon et tanins. Nous avons observé un effet des espèces sur la composition chimique des glands, en particulier pour la teneur en protéines, qui était de 83,2 g / kgMS et de 39,8 g / kgMS pour la graine de* Q. suber *et de* Q. rotundifolia*, respectivement. La date de récolte a affecté la teneur en tanins, qui a diminué au cours du processus de maturation comme précédemment observé par d'autres auteurs.*

Mots-clés. *Gland – Composition chimique* – Quercus rotundifolia – Quercus suber – *Date de la récolte.*

I – Introduction

Acorns from *Quercus rotundifolia* and *Quercus suber* are, together with pasture, the main feed resources available for the fattening period of Alentejano and Mediterranean pigs in the traditional silvo-pastoral system – "Montanheira" or "Montanera" in Portugal and Spain, respectively. Ripening and shedding of acorns occurs between October and February making the fruits available on the ground to be ingested by the pigs. The acorn chemical composition may vary depending on *Quercus* species and variety, site of production and fruit insect infestation (Gea-Izquierdo *et al*., 2006), among other factors. Shedding date, as a result of fruit

maturation, and or climacteric conditions such as hydric stress and strong winds, may also affect chemical composition of acorns and consequently its nutritive value. The chemical composition of acorns has been referred, together with pasture availability, as a determinant factor to the superior quality of meat and meat products from Alentejano and Iberian pigs (López-Bote, 1998; see Tirapicos). "Montanheiras" are carried out, both in *Q. suber*, *Q. rotundifolia* or mixed stands from October to February. Therefore a better knowledge of the chemical composition of acorns associated with these different conditions may contribute to a more rational utilization of "Montanheiras".

The aim of this study was to evaluate the effect of species (*Q. suber* or *Q. rotundifolia*) and time of shedding on the chemical composition of acorns.

II – Material and methods

Eight trees, (four *Q. rotundifolia* and four *Q. suber*) were randomly selected on a mixed oak stand in the Herdade da Mitra (Alentejo, Portugal). A square of about 2 m^2 was defined on the ground, underneath each canopy (crown area) and cleaned with a hand-rake in order to remove all acorns present. Collection of samples (about 500 g) was carried out every fortnight, starting from the day of the first cleaning of the squares. Immediately after each sample collection, the area of the defined squares was cleaned to ensure that each sample contained only acorns fallen during the previous 15 days. Six sample collections were carried out on October the 13th and 27th, November the 10th and 24th and December the 9th and 22nd of 2008. After collection from the ground, the acorns were taken to the laboratory, and a subsample of about 100 acorns was weighed to calculate the average acorn weight. The acorns were than separated into hulls and kernels using a knife and the weight of each portion recorded. Hulls and kernels were frozen at -20 ºC, freeze dried and ground (Ø1mm). Samples of the kernels were analyzed for dry matter (DM) (103º C with forced ventilation for 24 h), ashes (charred followed by combustion in a muffle furnace at 550 ºC), protein by the Dumas method (AOAC, 2005) in a LECO system (FP528), crude fat in a Soxtec system (AOAC, 2005) and neutral detergent fiber (NDF) by the method proposed by Goering and Van Soest (1970) and modified by Van Soest and Robertson (1980). Starch content of kernels was determined using an enzymatic method (kit MEGAZYME K-TSTA 04/2009) as described in AOAC, 2000. Phenolic compounds were extracted from the kernels (100 mg) twice, using an acetone solution at 70% (10 and 5 ml for the first and second extraction respectively). Samples with the acetone solution were sonnicated (20min) at 4 ºC and after centrifuged (3000xg for 10 min at 4 ºC). The supernatants were removed and total phenolic compounds (TPC) were measured using the Folin-Cicaulteau method as described by Makkar *et al*. (1993). Extractable tannins (ET) were calculated indirectly after measuring the non-precipitable phenols (phenols other than tannins, NPP) using polyvinyl polypyrrolidone (PVPP). NPP were left in solution after adding polyvinyl polypyrrolidone (PVPP) to the original extract, which precipitates along with the tannins, and measured as the TPC. ET were calculated as follows: ET=TPC-NPP. The results obtained were submitted to a 2x6 ANOVA (factor 1: oak species; factor 2: shedding period) using the statistics software package SPSS 17.0. Normality of distributions and homogeneity of variances were guaranteed.

III – Results and discussion

Fresh acorn (from Q. *rotundifolia* and Q. *suber*) average weights and percentage of pulp along the six shedding periods are presented in Table 1. Acorn average fresh weight was quite variable, as shown by the sd's presented in Table 1, and ranged from 3.7 to 5.8 g which is within the values reported in the literature (Rodríguez-Estévez *et al*., 2008). Acorns from Q. *rotundifolia* were heavier and larger than those from Q. *suber* ($P<0.05$, Table 2). Although not significant ($P=0.078$) there was a trend to an increase in acorn size along the shedding periods which is in line with an increase ($P<0.05$) in the kernel percentage of the fruits along those

periods. The effect of species and shedding period on the physical characteristic of acorns might be relevant for the choice process of the animals for the available acorns, since pigs have been shown to prefer larger acorns with a higher pulp percentage (Rodríguez-Estévez et al., 2008).

Table 1. Physical characteristics of acorns from *Q. rotundifolia* and *Q. suber* along 6 shedding periods (mean±sd)

		Shedding period						
	Acorn	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Acorn weight (g)	*Q. r.*	3.9±1.5	4.7±1.0	5.5±1.7	5.8±0.8	5.7±0.5	5.3±0.4	5.1
	Q. s.	3.9±0.7	3.7±0.6	4.1±0.2	4.9±1.1	4.8±1.3	4.1±1.4	4.3
	Total	3.9	4.2	4.8	5.3	5.3	4.7	4.7
Acorn pulp (%)	*Q. r*	64.7±10.9	69.9±12.2	75.1±9.0	79.4±4.0	77.9±1.4	78.3±1.3	74.2
	Q. s	65.1±8.5	65.0±10.2	74.9±5.2	79.7±1.6	80.3±1.8	81.1±3.6	74.3
	Total	64.9[a]	67.5[a]	75.0[ab]	79.6[b]	79.1[b]	79.7[b]	74.3

[a-c] Means within the same row with no common superscript are significantly different (P<0.05) according to Tukey's HSD test. *Q.r.: Quercus rotundifolia; Q.s.: Quercus suber.*

Table 2. Statistical parameters for the analysis of the effect of species and shedding period on the physical characteristics of acorns

	Effects								
	Species			**Shedding period**			**SpxShed.p**		
	Sig[1]	**SEM**[2]	**n**	**Sig**	**SEM**	**n**	**Sig**	**SEM**	**n**
Acorn weight (g)	0.01	0.22	24	0.078	0.38	8	0.836	0.55	48
Acorn pulp (%)	0.941	1.43	24	0.00	2.48	8	0.907	3.51	48

[1]Significance; [2] Standard error of the mean.

Chemical composition of acorn kernels from *Q. rotundifolia* and *Q. suber* along the six shedding periods is shown in Table 3. Data obtained is within the range of values previously presented in the literature (Gea-Izquierdo *et al*., 2006, Tirapicos Nunes, 2007).

Variable nutrient contents of acorns, even within the same species, have been reported by Gea-Izquierdo *et al*. (2006) and are also present in our data. Acorn species had a significant effect on its kernel chemical composition (Table 4). Overall, kernel from *Q. rotundifolia* had a lower content in ashes and a higher content in fat, NDF and starch than that from *Q. suber* (Table 3). Protein was the nutrient that showed the largest difference between species. Although still low in protein, Q. *suber* kernels may supply twice as much protein when compared to *Q. rotundifolia* (83.7 *vs* 39.4 g/kg DM), which might be important in the absence or lack of pasture under the "Montanheira" system. Dry matter content of kernels from *Q. suber* was lower (P<0.05), than that from *Q. rotundifolia*. This was probably due to the earlier stage of maturation evidenced by the presence of a larger proportion of green acorns for *Q. suber* than for *Q. rotundifolia* in the first two or three shedding periods. Shedding period also affected (p<0.05) the DM content of acorns which is in line with the observed increase in its major components, fat and starch. Dry matter content of acorns is also highly dependent on the environment humidity (Vázquez *et al*., 2001), however this parameter was not registered in the present work.

Shedding period did not affect ash and protein content of acorn kernels (Table 4). This is not in agreement with Carbonero *et al*., (2006) that observed an increase in protein content along the Montanheira period. Starch is the major constituent of acorn kernel and its concentration increased (P<0.05) as the "Montanheira" progressed. A significant effect (P<0.05) of shedding period was also observed on kernel fat and NDF content. Fat content was higher than that reported by other authors (Almeida *et al*., 1992; Carbonero *et al.*, 2006) but also increased during the "Montanheira" period (on average from 59.5 to 99.3 g/kg DM).

Table 3. Chemical composition of acorn kernel from Q. *rotundifolia* and Q. *suber* along 6 shedding periods

		Shedding period						
		1	**2**	**3**	**4**	**5**	**6**	**Total**
DM g/kg	*1*	472,8±98,8	551,2±99,1	599,8±33,2	627,5±17,1	600,9±20,6	594,8±15,5	574,5
	2	358,0±76,0	426,2±76,0	493,3±43,3	554,6±20,0	549,8±6,7	544,4±15,7	487,7
	T	415,4a	488,7ab	546,6bc	591,1c	575,4c	569,6bc	531,1
Ashes g/kgDM	*1*	21,4±1,5	20,7±2,1	21,1±1,6	21,8±1,7	19,4±3,1	21,9±0,9	21,1
	2	28,8±3,6	29,8±3,3	29,2±2,3	29,0±5,1	25,1±1,8	25,8±1,9	27,9
	T	25,1	25,6	25,1	25,4	22,3	23,9	24,5
Protein g/kgDM	*1*	37,7±5,0	37,7±6,0	35,6±6,7	42,4±6,3	43,4±6,3	39,6±6,0	39,4
	2	93,8±7,9	87,0±7,5	86,3±5,0	83,4±6,6	75,7±8,2	76,0±10,5	83,7
	T	65,8	62,3	60,9	62,9	59,6	57,8	61,6
Fat g/kgDM	*1*	83,8±31,9	96,8±31,8	135,1±16,8	122,0±6,0	114,6±12,2	116,3±18,0	111,4
	2	35,1±20,8	48,2±20,8	68,5±13,6	79,0±12,1	82,0±20,8	82,2±15,7	65,8
	T	59,5a	72,5ab	101,8c	100,5bc	98,3bc	99,3bc	88,6
NDF g/kgDM	*1*	45,0±4,1	55,0±12,5	73,9±3,1	56,9±19,1	50,1±9,5	50,6±4,9	55,3
	2	45,0±4,0	49,1±3,7	58,9±3,8	50,7±18,8	42,4±1,0	39,4±2,7	47,9
	T	45,0a	52,1a	66,4b	53,8ab	46,3a	45,0a	51,4
Starch g/kgDM	*1*	512,5±19,9	479,8±43,3	522,1±28,3	599,5±10,8	551,2±17,9	535,1±46,8	536,1
	2	220,3±19,4	357,0±69,0	410,9±25,9	536,0±28,9	508,7±44,3	510,4±44,3	430,8
	T	386,5a	418,4ab	466,5b	567,8c	530,0c	531,7c	483,5

[a,b] Means within the same row with no common superscript are significantly different (P<0,05) according to Tukey's test. 1*: Quercus rotundifolia;* 2*: Quercus suber*.

Concentrations of phenolic compounds measured as g of tannic acid equivalent per kg sample, are shown in Table 5. Extractable tannins represented, on average, 97% (ranging from 93-98%) of the total phenolic compounds measured, suggesting that only a very small proportion of those were non-precipitable phenols. Kernels from *Q. suber* had a higher concentration of phenolic compounds then those from *Q. rotundifolia*, furthermore, phenolic compounds concentration of both species decreased (P<0.05) with the progression of "Montanheira". This has also been reported by Almeida *et al*. (1992).

Table 4. Statistical parameters for the analysis of the effects of species and shedding period on the chemical composition of acorns

	Effects								
	Species			Shedding period			SpxShed.p		
	Sig[1]	SEM[2]	n	Sig	SEM	n	Sig	SEM	n
DM	0,00	11,14	24	0,00	19,30	8	0,61	27,29	48
Ashes	0,00	0,54	24	0,16	0,94	8	0,44	1,33	48
Protein	0,00	1,43	24	0,31	2,47	8	0,01	3,49	48
Fat	0,00	3,95	24	0,00	6,84	8	0,82	9,67	48
NDF	0,01	1,92	24	0,00	3,33	8	0,71	4,71	48
Starch	0,00	8,50	24	0,00	14,72	8	0,00	20,82	48

[1]Significance; [2] Standard error of the mean.

Table 5. Concentration of phenolic compounds (g standard equivalen/kg acorn kernel) on acorn kernel from Q. *rotundifolia* and Q. suber along 6 shedding periods

		Shedding period						
		1	2	3	4	5	6	Total
TPC[1]	*Q. rot*	57,5±10,2	42,8±10,1	37,7±3,4	28,7±2,6	33,3±4,7	38,1±5,1	39,7
	Q. suber	129,5±18,6	85,1±20,5	67,9±8,5	47,6±7,7	58,0±14,8	63,0±16,7	75,2
	Total	93,5a	63,9b	52,8bc	38,2c	45,7c	50,6bc	57,4
ET[2]	*Q. rot*	55,0±10,3	41,0±10,3	36,0±3,5	27,6±2,7	32,0±4,6	36,6±5,2	38,1
	Q. suber	126,3±18,9	83,2±18,3	66,5±8,3	46,4±7,6	56,5±14,6	61,5±16,4	73,4
	Total	90,7a	62,1b	51,3bc	37,0c	44,3c	49,0bc	55,7

[a-c] Means within the same row with no common superscript are significantly different (P<0,05) according to Tukey's HSD test; [1] Total phenolic compounds; [2] Extractable tannins.

Table 6.Statistical parameters for the analysis of the effects of species and shedding period on the chemical composition of acorns

	Effects								
	Species			Shedding period			SpxShed.p		
	Sig[1]	SEM[2]	n	Sig	SEM	n	Sig	SEM	n
TPC[3]	0,00	2,34	24	0,00	4,06	8	0,00	5,74	48
ET[4]	0,00	2,35	24	0,00	4,06	8	0,00	5,75	48

[1]Significance; [2] Standard error of the mean; [3] Total phenolic compounds; [4] Extractable tannins.

IV – Conclusions

Chemical composition of acorn kernels is quite variable and this can be observed even within the same species. Acorn species affect their chemical composition in that those from *Q. rotundifolia* have a lower content in ashes, protein and tannins and a higher content in fat, NDF and starch than that from *Q suber*. These results show the nutritional advantage of *Q. rotundifolia* acorns, especially in what concerns to its energetic value due to the higher concentrations in starch and fat. These differences in chemical composition together with the lower concentration on tannins may justify the commonly observed preference of pigs for *Q. rotundifolia* acorns. Shedding period also affected the chemical composition of acorn kernels. In fact, an improvement of the nutritive value was observed, with fat, NDF and starch content increasing and tannin content decreasing as the "Montanheira" progressed.

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