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Interannual variability and evolution during the *montanera* period of Holm oak (*Quercus rotundifolia* Lam.) acorns

D. Tejerina[1]*, S. García-Torres[1], M. Cabeza de Vaca[1], R. Cava[2] and F. M. Vázquez[1]

[1] *Junta de Extremadura. Vicepresidencia Segunda y Consejería de Economía, Comercio e Innovación. Dirección General de Innovación y Competitividad Empresarial. Centro de Investigación Agraria. Finca La Orden-Valdesequera Finca La Orden. Ctra. N-V, km 372. 06187 Guadajira (Badajoz). Spain*

[2] *Food Technology. Faculty of Veterinary Science. University of Extremadura. 10071 Cáceres. Spain*

Abstract

Eighty-eight samples of *Quercus rotundifolia* acorns were analyzed during three consecutive years (2005/06, 2006/07 and 2007/08) to determine variations in their morphology, sanitary condition, and nutritional composition throughout the same period of *montanera* (free-range swine-raising system of SW Iberia) and between different years of study. Results showed that morphological characteristics remained constant throughout *montanera* season. Fungal damage increased, and *Curculio* sp. and *Cydia* sp. pests decreased over the course of the *montanera* season. Dry matter content decreased in both kernel and pericarp ($P < 0.001$), while the crude fiber and protein contents were not modified. The ash, nitrogen-free extract, and fat contents, however, were also affected by sampling date within a *montanera* season. With respect to the interannual variability, variations ($P < 0.05$) were observed in all the variables studied. It is therefore confirmed that there is variability in the sanitary condition and nutritional composition of acorns within the same *montanera* season and between different years of study. These could explain the differences in the chemical characteristics of Iberian pig tissues from animals raised in different *montanera* seasons, and even during the same *montanera* season.

Additional key words: acorn sanitary condition, *dehesa*, feeding, Iberian pigs, proximate composition.

Resumen

Variabilidad interanual y evolución durante el periodo de montanera de bellotas de *Quercus rotundifolia* Lam.

Ochenta y ocho muestras de bellotas de *Quercus rotundifolia* fueron analizadas durante tres años consecutivos (2005/06, 2006/07 y 2007/08), para determinar variaciones en la morfología, estado sanitario y composición nutritiva de las mismas durante un mismo periodo de montanera y entre diferentes años de estudio. Los resultados mostraron que las características morfológicas permanecen constantes durante la montanera. El ataque debido a la presencia de hongos aumentó, mientras que el de los insectos *Curculio* sp. y *Cydia* sp. disminuyó durante la montanera. El contenido en materia seca, tanto de la semilla como del pericarpio, también disminuyó ($P < 0,001$), mientras que el contenido en fibra bruta y en proteína no sufrió modificaciones. Sin embargo, el contenido en cenizas, sustancias extractivas libres de nitrógeno y grasa también se vio afectado por la fecha de muestreo dentro de una montanera. Con respecto a la variabilidad interanual, se observaron variaciones ($P < 0,05$) en todas las variables estudiadas. Por tanto, se puede afirmar que existe variabilidad en el estado sanitario y la composición nutritiva de bellotas dentro de una misma montanera y entre diferentes años de estudio. Esto podría explicar las variaciones en las características químicas de los tejidos de cerdo ibéricos de animales criados en diferentes periodos de montanera, e incluso en una misma campaña.

Palabras clave adicionales: alimentación, cerdo Ibérico, composición nutritiva, dehesa, estado sanitario de la bellota.

Introduction

Species of the genus *Quercus* represent the fundamental arboreal element in the «dehesa» ecosystem typical of Southwestern Europe. It is a Mediterranean forest type in which various species of *Quercus* coexist, and the primitive understorey has been eliminated by man to favor the growth of annual grasses and legumi-

* Corresponding author: david.tejerina@juntaextremadura.net
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Abbreviations used: CF (crude fiber), CP (crude protein), DM (dry matter), MUFA (monounsaturated fatty acids), NFE (nitrogen-free extract), SEM (standard error of the mean).

nous herbs (Olea *et al.,* 1989; Devesa, 1991). In this ecosystem, pigs of a local breed (Iberian) are bred in a free-range system, with a diet based on natural resources (acorns and grass), from October-November until January-February. This period is known as *montanera*.

The «dehesa» and the production of acorns for the feeding of the Iberian pig are intimately linked. This is reflected in the Spanish legislation by the Royal Decree 1469/2007 of 2 November (BOE, 2007) in which the Ministry of Agriculture and Fisheries directly associates the Iberian pig with the «dehesa» ecosystem.

The great social acceptance of the products derived from this system is mainly due to the high quality of the end products that result from this linkage. This quality is chiefly the result of the monounsaturated fatty acids (MUFA) that the acorn contributes to Iberian pig meat (Muriel *et al*., 2002).

The analysis of fatty acid composition has been the evaluation method used in Spain since the beginning of the 1980s for the quality discrimination of these products. Today, this method is widely questioned in the Iberian pig sector, and has fallen into disuse. Indeed, the lipid profile of the Holm oak (*Quercus rotundifolia*) acorns in Spain varies enormously (Vázquez and Doncel, 2002), and there are marked interannual variations within a single livestock rearing zone (Vázquez *et al*., 2001).

Preliminary studies have considered the nutritional composition and digestibility of acorns (Vázquez, 1998a; López-Carrasco *et al*., 2005; Rey *et al*., 2006) of both *Q. rotundifolia* and *Q. suber* (cork oak). Up to now, however, there has been relatively little reference to how these live food resources change over time. The research that has been previously carried out includes studies on the variations observed during the *montanera* season in the acorns' nutritional composition (Paredes, 1998), phytosanitary quality (Almeida *et al*., 1992), and their total availability, which determines the duration of the *montanera* (Benito *et al*., 1997), as well as the livestock density that the system can support (Aparicio-Macarro, 1987).

The objective of the present work was to study the temporal evolution of the morphology, sanitary condition, and proximate composition of acorns of *Q. rotundifolia* in a range-farming estate located in Southwestern Europe during the *montanera* season (November-January), and their variability throughout three consecutive years.

Material and methods

Study area and acorn sampling

The study covered three consecutive *montanera* seasons (years 2005/06, 2006/07, and 2007/08) in a farm characterized as a typical «dehesa» ecosystem. The farm, known as «Valdesequera» (39°03'N, 6°50'W), is located at km 65 of the Cáceres-Badajoz highway («Nacional E-100») in the southwest of the Iberian Peninsula.

Valdesequera's trees are Holm oak (100%), with a 0.3-0.5% cover fraction, *i.e.*, the fraction of ground area shaded by the vertical projection of the trees' outermost perimeters (measured as per one, units). The soil is a vertisol with a sandy texture and a pH of 5.6-6.7.

Samples were collected fortnightly during each of the *montanera* seasons (Table 1). For each sample, 2-3 kg of acorns were collected directly from the ground from at least 10 different randomly selected trees per plot (1 kg = 60-110 acorns).

The sampling areas were also randomly selected, and were isolated from the domestic animals bred in the farm. Wild birds could not be fully controlled, but farmers avoided their presence during the day. Acorns were collected directly from the soil and they were not cleared between each sample collection in order to reproduce as closely as possible the reality of the Iberian pig's *montanera* season. This was important to determine the real variation in the nutritive composition and sanitary condition of acorns, since variation could be due to the ripening stage during the three months of the *montanera* season. Furthermore, it could be the reason for the great variability in the quality of meat products between different years or within the same year.

The heterogeneity in the number of samples for each year was the unavoidable result of the availability of acorns and the duration of the *montanera*, both of which

Table 1. Experimental design: sampling distribution throughout the three years of the study

Date	2005/2006	206/2007	2007/2008	Total samples
	Number of samples			
15-30 November	7	6	8	21
1-15 December	6	8	8	22
15-30 December	6	6	8	20
1-15 January	6	8	8	22
15-30 January	0	0	3	3
Total	25	28	35	88

Table 2. Meteorological data (temperature and rainfall) for the period of study

Year	Temperature (°C)		Rainfall (mm)		
	Maximum	Mean	Minimum	Monthly rainfall	Effective rainfall[1]
2005/2006	13.5	6.4	1.2	36	0.5
2006/2007	14.2	7.3	3.4	42	0.7
2007/2008	14.0	8.5	1.3	57	0.8

[1] Effective rainfall is the daily rainfall minus the losses from evaporation and runoff. It is the real amount of water that is usable by plants, and is calculated by the method proposed by the Soil Conservation Service (SCS) of the US Department of Agriculture (USDA). In this case, it is approximately 45% of the daily rainfall.

depend on such uncontrollable factors as weather conditions (Table 2) or the presence of wildlife feeding on the acorns. The total production of acorns was estimated following the method proposed by Vázquez (1998b).

Acorn analyses

After collection, samples were taken to the laboratory of the «La Orden-Valdesequera» research center of the regional government of Extremadura, for their analysis. The morphological, sanitary, and moisture content characterization was performed on the fresh material, which was then stored frozen at –20°C for the subsequent determination of nutritional parameters.

From each sample batch, 25 acorns were selected at random. After removing the acorn from its cupule of hardened bracts, the length (mm) and width (mm) were measured using an Electronic Digital Calliper (Mod. 5900603, COMECTA®). The length was taken from the point of the base to the apex, excluding the final appendix, and the width was taken at the widest part of the acorn.

For each sampling point, 50 acorns were chosen at random. The kernel of each acorn was opened, and the percentage of acorns attacked by phytophagous insects (*Curculio* sp., *Cydia* sp.) or fungus (*Penicillium* sp., *Aspergillus* sp.) was determined visually, and guided by photographic standards to make the evaluation and classification of each type of damage easier (see Soria *et al.*, 1999). The acorn was considered as damaged if there was any indication of eggs, larvae, the presence of the insect, putrefaction, etc. The results were expressed as percentage of unhealthy acorns relative to the total number of acorns.

For each sampling point, 5 subsamples of 10 acorns each were taken, and the pericarp was removed. The dry matter (DM) content was assayed following the official AOAC method (AOAC, 2003) were fresh acorns are oven-dried at 105°C. The results are expressed as g kg^{-1} fresh-acorn.

Proteins, ash, and crude fiber (CF) were also assayed following the official AOAC methods (AOAC, 2003) applied to the dry-milled sample. The results are expressed as g kg^{-1} DM. The fat content was assayed following the method described by Folch *et al*. (1957), again applied to the dry-milled sample. The results are expressed as g kg^{-1} DM.

The total NFE (nitrogen-free extract) content was calculated following the Weende method (Henneberg and Stohmann, 1867) using the following equation:

$$\text{g NFE/kg DM} = [1000 - (\text{g crude protein/kg DM} + \\ + \text{g fat/kg DM} + \text{g ash/kg DM} + \\ + \text{g crude fiber/kg DM})]$$

Statistical analysis

The statistical analysis of the data consisted of a multiple analysis of variance (two-way ANOVA) using the software package SPSS.PC+ (2005), taking into account the sampling date (evolution) within a given period, the year effect, and the interaction of the two (sampling date × year effect). Differences were compared using the means. Tukey's HSD test was applied to compare the mean values of each group. Statistical significance was set at $P < 0.05$.

Results

Morphology (length and width) of the acorns

The morphological characteristics, length and width (Table 3) did not show significant variations with

Table 3. Effect of date and year of sampling on morphology, sanitary condition and proximate composition of acorn samples

Variables	Date of sampling					Year of sampling			SEM[1]	Significance level[2]		
	15-30 Nov	1-15 Dec	16-31 Dec	1-15 Jan	16-31 Jan	2005/06 (n = 25)	2006/07 (n = 28)	2007/08 (n = 35)		Sampling date	Year	Inter-action
Morphology												
Length (mm)	37	37	37	39	38	36^a	38^b	38^b	0.3	ns	**	***
Width (mm)	16	17	16	16	16	15^a	17^b	17^b	0.1	ns	**	ns
Phytosanitary status[3]												
% Curculio sp.	8.3^b	2.8^a	2.0^a	2.8^a	6.8^{ab}	2.7^a	1.6^a	8.2^b	0.79	***	***	***
% Cydia sp.	5.3^b	1.8^a	1.4^a	1.0^a	0.8^a	1.0^a	1.9^a	3.5^b	0.44	***	**	***
% Fungus	7.9^{ab}	4.5^a	10.5^{ab}	14.7^b	17.0^b	6.8^a	6.4^a	17.4^b	1.27	**	***	ns
Proximate composition												
DM kernel (g kg^{-1})[4]	586^a	607^{ab}	607^{ab}	630^{bc}	639^c	605^b	588^a	638^c	3.6	***	***	*
DM pericarp (g kg^{-1})	608^a	613^a	621^{ab}	647^{ab}	640^b	645^c	602^a	623^b	4.0	***	***	***
Crude protein (g kg^{-1} DM)[5]	66	62	65	63	68	45^a	58^b	89^c	2.2	ns	***	ns
Fat (g kg^{-1} DM)	70^a	71^a	79^b	73^{ab}	78^b	64^a	76^b	76^b	1.4	**	***	ns
NFE (g kg^{-1} DM)	794^b	767^{ab}	769^{ab}	788^{ab}	755^a	878^c	802^b	660^a	11.5	**	***	***
Crude fibre (g kg^{-1} DM)	81	85	84	77	86	53^a	69^b	120^c	3.6	ns	***	ns
Ash (g kg^{-1} DM)	26^{ab}	25^a	26^b	27^b	29^c	27^b	20^a	31^c	0.6	***	***	***

[1] Standard error of the mean. [2] ns: not significant ($P > 0.05$). *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$). [3] Values are % unhealthy acorns [4] Values expressed as g DM kg^{-1} fresh acorns. [5] Values expressed as g kg^{-1} dry matter of acorns. Means with the same letters (a,b,c) indicate homogeneous subsets for $P = 0.05$ according to Tukey's HSD test.

regard to the sampling date ($P > 0.05$) throughout the *montanera* season. However, there were statistically significant interannual variations in both length and width ($P < 0.01$). In this sense, the acorns of the 2005/06 season were generally shorter than those of the next two years (2006/07 and 2007/08). The greatest average widths corresponded to the acorns of 2007/08, with those of 2006/07 being intermediate in width, and those of 2005/06 of the smallest widths.

The average length of the acorns was also influenced by the interaction between sampling date and study year ($P < 0.001$).

Acorn production estimation was 366 kg ha $^{-1}$, 427 kg ha $^{-1}$, and 415 kg ha $^{-1}$ for the 2005/06, 2006/07, and 2007/08 seasons, respectively.

Sanitary condition of the acorns

The percentage of unhealthy acorns due to the presence of *Curculio* sp. and *Cydia* sp. varied throughout *montanera* season ($P < 0.001$) (Table 3), being greater at the beginning and lower at the end of the season. In contrast, the percentage of acorns attacked by fungi was greater in the samples at the end than at the beginning of the *montanera* season ($P < 0.01$).

There were interannual variations in the proportion of unhealthy acorns. This was highly significant ($P < 0.001$) with regard to the presence of both *Curculio* sp. and fungi, and very significant ($P < 0.01$) in the case of *Cydia* sp. (Table 3). The year with the greatest incidence of these parasites was 2007/08, with 2005/06 and 2006/07 enjoying a greater proportion of healthy acorns.

The interaction between the sampling date and the effect of the sampling year significantly influenced the presence of *Curculio* sp. and *Cydia* sp. This has an overall impact on the final quality of the acorns in each *montanera*.

Proximate composition of the acorns

The proximate composition of the acorns is shown in Table 3. Dry matter content of both the kernel and the pericarp varied significantly ($P < 0.001$), both throughout the different years and during the *montanera* season, the highest contents being observed at the last stage. Similarly, fat and ash contents also changed during the *montanera* season ($P < 0.01$), and the highest content were also observed in the final stages. In contrast, the NFE declined throughout the *montanera* season ($P < 0.01$). The protein and fiber contents, however, were unaffected by the sampling date during a *montanera* season ($P > 0.05$).

There was a noticeable effect of the sampling year on the concentrations of these parameters, with significant variations observed in all of them ($P < 0.001$). These variations mean that the final quality of the acorns differed between the three years of the study.

The interaction of the year effect and the sampling date in a given year directly affected the NFE, ash, and mean dry matter content of the pericarp ($P < 0.001$), and to a lesser extent, the dry matter content of the kernel ($P < 0.05$).

Discussion

Morphology (length and width) of the acorns

There were no differences in the length and width of the acorns within a *montanera* season. This was expected since, during the fruiting period, the trees produce acorns of certain dimensions which may vary from tree to tree (Afzal-Rafii *et al.*, 1992). But since acorns then fall and mature on the ground, they cease to receive nutrients from the tree and these dimensions do not change (Vázquez, 2000). Both the data collected for length and width in the present study are coherent with the literature values reported by Vázquez *et al.* (1992) in a research regarding the characterization of acorns from *Q. rotundifolia* Lam. in Extremadura (Spain).

There were, however, interannual differences in these parameters. This may have been due mainly to each year's meteorological conditions (see Table 2). The variations in rainfall and temperature would have conditioned the soil properties, affecting the availability of nutrients for the trees, and therefore their fruiting capacity. A year with unfavorable weather conditions would lead to the trees being stressed, thus affecting the amount and size of the fruit they produce. As a matter of fact, this is a clear indication of the interannual variability of acorns in a given geographical zone (Almeida *et al.*, 1992; Vázquez *et al.*, 2001). In the year 2005/06, the total acorn production was lower and the acorns were smaller, in both length and width, than in the other two years of study. And indeed, the meteorological parameters for that year (Table 2) were the most unfavorable, with the lowest temperatures and the least rainfall. These conditions probably put the trees under greater stress, affecting the total production and the size of the fruit, since the trees would have had insufficient nutrients to produce larger fruit.

Sanitary condition of the acorns

During a *montanera* season, the percentage of acorns attacked by *Curculio* sp. and *Cydia* sp. was lowest during the final stages, whereas the presence of fungi was highest during this same period. This inverse relationship between the two types of attack is consistent with the explanation given by Vázquez (2000) that the greater presence of phytophagous insects in the early *montanera* stages facilitates subsequent fungal attack within the seeds (Vázquez, 1998a). Moreover, phytophage attacks occur when the acorns are still on the tree, and the larvae continue their life cycle while the seed matures on the ground. In contrast, fungi penetrate into the interior when the acorns are already on the ground, and therefore the presence of moisture favors their proliferation (Vázquez, 2000).

The differences observed in the three years of study may have also been due, besides the meteorological conditions, to a greater or lesser presence of plagues of phytophagous insects in the zone, since these have been observed to negatively influence the total acorn production and the phytosanitary quality of acorns (Vázquez, 1998a; Siscart *et al.*, 1999; López-Carrasco *et al.,* 2004).

A greater incidence of phytophages and fungi was found in the 2007/08 *montanera*, the year in which the percentage of unhealthy acorns was the highest. This may have been because the average temperature was higher during that year, thus favoring an increased presence of phytophagous insects. Furthermore, the average rainfall was also higher, favoring a greater proliferation of fungi.

All this confirms the variability in the phytosanitary quality of acorns during the *montanera*, as has been reported in other works (Almeida *et al.*, 1992; Paredes, 1998; Vázquez, 1998a). A study of the quality of acorns and its impact on the quality of Iberian pig meat found that the latter indeed depends on the phytosanitary status of the acorns (Daza *et al.*, 2007).

Proximate composition of the acorns

The acorn is a dynamic food source whose proximate composition evolves as the seed matures and in response to external factors such as climate and the presence of parasites (Almeida *et al.,* 1992; Vázquez, 2000). During a montanera season, variability in its nutritive composition and sanitary condition could be due to two mechanisms: new acorns that fall into the ground and pre-existing acorns that are kept for long periods, with different levels of ripening.

Thus, a real tendency could be observed in the results presented in Table 3. As the acorns ripen on the ground during the *montanera* season, changes take place in their proximate composition, mainly in the dry matter content of both the kernel and the pericarp, and in the ash, fat, and NFE contents. This is because once the acorns have fallen to the ground they are subject —more or less simultaneously— to a series of, at times contrasting, processes of ripening, germination, desiccation, dampening, rotting, and attack by pests. Since all these processes can lead to changes in the acorns' physical (weight) and organoleptic (external aspect, smell, flavor) characteristics and in their proximate composition, there would thus be a continual addition of new elements to the variability experienced by the acorns that are affected by these processes, especially at the end of the *montanera* season (Almeida and Baptista, 1992; Fernández *et al.,* 2004; López-Carrasco *et al*., 2004). These biochemical processes (especially germination) consume carbohydrates as fuel, which is why a decline in the NFE content was observed as the ripening and germination processes took place during the *montanera* season.

Furthermore, the ripening process on the ground itself causes a progressive loss of moisture, which would be reflected in the increasing DM content of both the kernel and the pericarp, and an even greater concentration of minerals. Nevertheless, desiccation is not a clear and constant process, but depends on the environmental humidity and the soil moisture during the *montanera* months, and on other factors such as adjacent vegetation, which may protect the acorns from inclement weather (Vázquez, 2000).

Variability in the fat content may be due to the presence or absence of phytophagous pests, as López-Carrasco *et al.* (2004) found that the crude fat of the kernel is reduced in acorns attacked by *Curculio* sp. Thus, at the beginning of the *montanera* season, when there was a greater incidence of this pest in the kernels, the fat content was lower. In agreement with the observations of Almeida and Baptista (1992), Fernández *et al.* (2004), López-Carrasco *et al*. (2004) and Olea *et al.* (2004) we did not observe changes in the protein and fiber contents.

The amount of feed available decreased over the course of the *montanera* as a consequence of the attack by phytophages and adverse weather conditions. However, acorns are a highly energetic resource and have large amounts of protein. This situation allows for two different uses of the *montanera*: i) the first half of the *montanera* season, with plenty of protein-rich food from acorns, which is useful for the growth of the Iberian pigs; and ii) the second half of *montanera* season, with a lower food availability, originating in acorns from the top of *Quercus* trees and with a higher energy content, intended for fattening of the Iberian pigs.

The interannual differences in the parameters studied (see Table 3) could be due to the fact that acorns, like any other fruit, receive their nourishment from the tree, and their chemical characteristics will therefore be influenced by factors such as the greater or lesser incidence of parasites, or by the meteorological conditions (Almeida, 1992; Vázquez, 2000; Vázquez *et al*., 2001). The latter will affect the soil's characteristics and the availability of nutrients that can be taken up by the tree, thus affecting the tree's fruiting capacity and the total acorn production.

Overall, chemical composition (dry matter, crude protein, fiber and fat contents) agreed with the range of values found in the literature (Cava *et al*., 1997; Daza *et al*., 2005; Rey *et al*., 2006) with slight variations depending upon the season studied. Results evidenced the marked differences in the proximate composition of acorns in different years of study in the same geographical area, as well as in studies in other distinct geographical areas, as has been described in the work by Almeida *et al*. (1992), Vázquez (2000) and Rodríguez-Estévez *et al*. (2008).

Variations between *montanera* seasons and over the course of a *montanera* with regard to the morphological characteristics, health state, and proximate composition of acorns are relevant factors that affect their consumption by Iberian pigs, thus affecting the final characteristics of their tissues. Recently, Rodríguez-Estévez *et al*. (2009) described a selective consumption by Iberian pigs for acorns with larger kernels, and a variation in acorn preferences throughout the *montanera* period. Additionally, the acorns' state of conservation (pest attacks or rotting) has been described to be a determinant factor on the preference of consumption that further affects their chemical composition. (Steele *et al*., 1996; Rodríguez-Estévez *et al*., 2009). In this sense, López Carrasco *et al.* (2005) and Daza *et al*. (2007) reported that *Curculio* sp. infestation affects the acorns' dry matter, crude protein, fat, and crude fiber contents, as well as their fatty acids profiles. These alterations consequently modified the fat content and the fatty acids profiles of Iberian pigs. These authors reported that the consumption of healthy acorns by pigs produced a higher intramuscular fat percentage in *Longissimus dorsi* than in pigs fed with acorns infested with *Curculio sp*. Additionally, the subcutaneous backfat, and the intramuscular and liver fat from pigs fed healthy acorns had higher C18:1n-9 and MUFA, and lower C18:0 and SFA proportions than those of pigs fed acorns infested with *Curculio* sp. These facts confirm that the quality of acorns (size, health state and composition) is not homogeneous and varies over the years and over the course of *montanera* seasons. These variations could be responsible for differences in the fat content and the fatty acid profiles of the tissues of pigs reared in different *montanera* seasons and even from the same *montanera* season.

In conclusion, the present results have shown that the acorn, as a live material, undergoes a series of changes in its sanitary condition and nutritional composition throughout its ripening stage, which will influence its final quality.

The changes observed during the *montanera* period and the differences between the three years of study demonstrate that the acorn is a dynamic and variable element of the «dehesa» ecosystem. These dynamic changes could be the explanation for the variability in the tissue composition of Iberian pigs and therefore on the quality of meat products from Iberian pigs raised under this unique free-range system.

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