

PROCESSING AND PRODUCTS

Nutritional value of meat lipid fraction from red-legged partridge (*Alectoris rufa*) obtained from wild and farmed specimens¹

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ABSTRACT The red-legged partridge (*Alectoris rufa*) is a feathered game species of great socioeconomic importance in its native range and also in the UK. The aim of this study was to present a detailed comparison of meat's lipid fraction obtained from wild and farm-raised specimens and simultaneously compare the breast and leg meat portions.

Meat from wild specimens had a significant ($P < 0.05$) lower proportion of saturated fatty acid (less 5.1%) and presented better P/S and n-6/n-3 ratios, and atherogenicity index than farm-raised counterparts. The wild specimens presented significant ($P < 0.001$) higher contents of total vitamin E (8.8 vs. 2.2 $\mu\text{g/g}$ of

fresh meat), is for that reason less prone to lipid peroxidation than farm-raised specimens.

Meat portions differed significantly ($P < 0.05$) on total lipid and total cholesterol contents and in all partial sums of fatty acids. The breast was leaner (0.86 vs. 1.47 g/100 g of meat), with lower total cholesterol (37.5 vs. 54.7 mg/100 g of meat), lower saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid, and n-6 polyunsaturated fatty acid (less 0.27, 0.28, 0.10, and 0.11 g/100 g of fresh meat, correspondingly). Regarding the fatty acid ratios and lipid quality indexes, breast meat presents better n-6/n-3 ratio and atherogenicity and thrombogenicity indexes.

Key words: *Alectoris rufa*, red-legged partridge, fatty acid profile, total cholesterol, vitamin E

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INTRODUCTION

Partridge is a common name given to several species belonging to 14 genera within the Phasianidae family. In Europe, four different species are regarded as gamebirds, the gray partridge (*Perdix perdix*), the red-legged partridge (*Alectoris rufa*), the rock partridge (*Alectoris graeca*) and the chukar partridge (*Alectoris chukar*). Partridges are hunted throughout Europe, and it has been estimated that more than 7.5 million partridges were shot dead in a single hunting season (CABS, 2015). Among them, the red-legged partridge was the most hunted species in Europe, more than 5 million specimens, representing 66.4% of the European global partridge hunting bag (CABS, 2015).

The red-legged partridge is an important gamebird with high socioeconomic value in the rural areas of its native range (Portugal, Spain, France, and

Italy) and also in the UK, where it was introduced in the XVIII century (Arroyo et al., 2011; Birdlife International, 2017). In Spain, almost 12.7 million gamebirds were shot dead in the year 2014, of which nearly 2.7 million were red-legged partridges, with a market value of 5.3 billion euros (Ministerio de Agricultura, 2015). In Portugal, the number is quite lower but in the hunting season of 2016/2017, more than 220 hundred thousand red-legged partridges were shot dead.

Populations of wild red-legged partridges have experienced an important decline in recent decades all over the Iberian peninsula, which has been followed by a concomitant increase in the release of farm-reared specimens (Casas et al., 2016). Bird releases for restocking is a widespread game management action in Europe (Duarte and Vargas, 2004; Sokos et al., 2008). In Portugal, the rearing of the red-legged partridge in specialized farms is under the regulation of the national laws and under the control of the authority of wildlife conservation (ICNF). Periodically, red-legged partridges from these specialized farms are collected in the rearing facilities to evaluate their phenotype compliance with the wild species and their genetic purity, to avoid hybridization with other species within the

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Alectoris genus. The sanitary condition of these birds is under periodic surveillance and bird welfare is also monitored throughout the production cycle. In Spain and the UK, it has been estimated that more than 4 and 6.5 million farm-reared specimens, correspondingly, are released on an annual basis (Bicknell et al., 2010; Caro et al., 2015).

Nowadays, game meat is a freely available commodity of increasing popularity in western societies, representing an added diversity to human's diet (Quaresma et al., 2016), being regarded as a highly valued food (Hofbauer et al., 2010), and that is also the case of red-legged partridge, which is highly appreciated by Iberian consumers (Casas et al., 2016). However, due to the decline in wild flocks and the increased use of farm-reared partridges for both releases and driven-shooting, it is expectable that most of the red-legged partridge meat entering the market is originated from farm-reared birds rather than from wild specimens.

Among Iberian hunters, there is a generalized opinion that farm-raised individuals are of inferior quality to wild ones (Negro et al., 2001), they claim that the differences began to be observed in the field (since the wild partridge is much more stealthy, faster runner, and more efficient flyer than farmed partridges), and ends in the dish (different flavor and sensorial characteristics).

Considering the importance of intramuscular fat content and composition on meat sensorial and nutritional quality, the present study was conducted to provide a detailed characterization of red-legged partridge meat lipid fraction, and the comparison of wild and farmed specimens (raised under semi-extensive conditions). Such comparison encloses detailed information on the lipid fraction of breast and leg meats, comprising the total lipids (TL), total cholesterol (TC) contents, fatty acid (FA) and vitamin E profiles, FA ratios, and lipid quality indexes.

MATERIAL AND METHODS

Chemicals and Reagents

High-performance (high-pressure) liquid chromatography (HPLC) grade n-hexane was purchased from Merck (Darmstadt, Germany); absolute ethanol (99.8% v/v) from AGA (Lisboa, Portugal); and 1,4-dioxane was from Fluka (Madrid, Spain). High-purity gases, R grade (helium, hydrogen, nitrogen, and air), were acquired from Air Liquide (Lisboa, Portugal).

A mixture of 37 fatty acids methyl esters (FAME) was acquired from Supelco (Bellefont, PA, USA). For vitamin E analysis, standard solutions of tocopherols (α , β , γ , and δ) and tocotrienols (α , β , γ , and δ) were purchased from Calbiochem (La Jolla, California, USA) and were prepared and diluted in n-hexane. Purified water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals used were of analytical grade. Fourteen percent Boron-trifluoride in methanol, and cholesterol

Table 1. Composition of the concentrate feeding provided to red-legged partridge chicks during different growing periods.

Concentrate Age group	I			II			III		
	<3rd wk	>3th and <9th wk		>3th and <9th wk		>9th and <12th wk		>9th and <12th wk	
Crude protein	32.0%	28.0%		28.0%		22.0%		22.0%	
Crude fiber	3.8%	4.7%		4.7%		6.2%		6.2%	
Crude fat	3.2%	2.9%		2.9%		3.4%		3.4%	
Crude ash	7.3%	8.2%		8.2%		8.5%		8.5%	
Lysine	1.5%	1.3%		1.3%		0.9%		0.9%	
Methionine	0.6%	0.6%		0.6%		0.4%		0.4%	
Calcium	1.3%	1.1%		1.1%		1.2%		1.2%	
Sodium	0.1%	0.2%		0.2%		0.2%		0.2%	
Phosphorus	0.9%	0.7%		0.7%		0.7%		0.7%	

standard (>99.0% purity) were acquired from Sigma-Aldrich (Steinheim, Germany), and all other chemicals used were of analytical grade.

Birds and Sampling

This study used a total of 28 red-legged partridges, 14 farm-raised specimens (7 from each sex), and 14 wild specimens (half of each sex). The farmed red-legged partridge (*Alectoris rufa*) used in this study was provided by CAÇABRAVA (Santa Cita, Portugal), a company specialized in gamebird rearing for hunting purposes. The production of red-legged partridge was performed in full compliance with national laws (Official Journal of the Republic of Portugal, law 202/2004 18th of August and Ordinance 464/2001 8th of May). All rearing steps are performed with the minimum contact to humans to assure birds' feral condition.

The rearing facilities are original and were designed for gamebirds and adapted to the specificities of the species. Sheds are composed by 3 different zones: (1) brooder barn, (2) night shelter, and (3) rearing pen. Each one is adapted to the growing stage requirements, and was planned to prepare the birds to life in their natural habitat. After hatching, chicks stay in the brooder barn during the first 6 wk of life. Then they pass to the adaption zone (night shelter), where they remain until 10 to 12 wk of age. During this period, juveniles continue their development and adaptation to weather conditions, since this area makes frontier with the rearing pen, being separated from it just by a physical barrier made with net door. The rearing pen is prepared to resemble the habitat where birds will be further released, displaying some trees, several bushes in earth floor, with some rocky areas. Feed and water are only available in the night shelter. The limited access to feed and water forces birds to enter the night shelter, facilitating their capture.

Considering the nutritional requirements of the species, in the first 12 wk of life chicks were fed with a commercial compound feed mixture, especially developed for the red-legged partridge and adapted to their age nutritional requirements (Provimi, Portugal; the composition is described in Table 1). From the 12th to the 15th wk, the compound feed mixture was progressively replaced by a mixture containing wheat, oat,

maize, sorghum, rye, and sunflower seeds in similar weight proportion.

The wild red-legged partridges ($n = 14$) used in the study were provided by FENCAÇA (Portuguese National Federation of Hunting). To assure red-legged partridges' wild condition, birds used in the study were hunted in reserves not using the release of farm-raised red-legged partridges throughout the last 5 yr.

For this study, farmed red-legged partridges were randomly collected among shot birds from seven driven shooting hunting journeys (to provide one male and one female per hunting journey). The specimens used in the study were representative of seven different flocks, since birds from a single flock are all used in one driven shooting journey. After collection, red-legged partridges were kept in refrigeration ($<5^{\circ}\text{C}$) until processing (an overall period of 16–18 h). The wild red-legged partridges used on the study were shot in the morning period taken home by the hunters and stored in refrigeration. These birds were collected 1 d after they were shot dead, and transported to the laboratory in refrigeration ($<5^{\circ}\text{C}$).

In the laboratory, the red-legged partridges were weighted, plucked, and skinned. Breast and leg muscles were collected from both sides. Breast muscles were trimmed of external fat depots and visible connective tissues, whereas legs were firstly deboned and then trimmed. Breast and leg meat samples were individual and separately grinded in a food processor (Moulinex, France). Subsequently, half of each meat portion was vacuum packed and frozen at -70°C until analysis, while the remaining portion was frozen, lyophilized (-60°C and 2.0 hPa; Edwards High Vacuum International, UK), and maintained desiccated at room temperature, until analysis.

Analytical Methods

Meat TL content was determined gravimetrically (duplicate values accepted for coefficient of variation $<5\%$) from lyophilized meat samples (0.25 g) using a procedure previously described by Folch et al. (1957). To obtain the FA composition, direct transesterification of intramuscular fat was performed according to Rule et al. (1997). Briefly, 100 mg of lyophilized muscle and 100 μl of internal standard solution (2 mg/ml nonadecanoic acid in n-hexane) were mixed with 2 ml of 14% boron-trifluoride in methanol, then homogenized using a vortex, and placed in a water bath at 80°C for 2 h with stirring. FAME were analyzed by gas-liquid chromatography using a Shimadzu GC-2010 Plus (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. A BPX-70 capillary column (60 m, 0.25 mm i.e., 0.25 μm film thickness, SGE Chromatography Supplies, Austin, TX) was used for FAME separation, using helium as the carrier gas at a flow rate of 1 ml/min, and a temperature gradient: 50°C for 1 min, increased at $50^{\circ}\text{C}/\text{min}$ to 150°C and held for 1 min, increased again at $1^{\circ}\text{C}/\text{min}$ to 200°C , held for 2 min, and finally increased at $3^{\circ}\text{C}/\text{min}$ to 220°C . The injector and detec-

tor temperatures were maintained at 250°C . One microliter of sample was injected. Identification of FAME was achieved by comparison of their retention times with authentic standards.

The determination of tocopherols, tocotrienols, and TC was performed as previously described by Prates et al. (2006). The chromatographic analysis was performed by HPLC, using an integrated HPLC Jasco System (Jasco, Japan) equipped with a PU-2089 pump, an AS-2057 automated injector, an FP-2020 fluorescence detector, and an MD-2018 multi-wavelength diode array detector. Tocopherols and tocotrienols were detected/quantified by fluorescence (wavelength excitation at 290 and emission at 330 nm), while cholesterol was detected/quantified by UV-Vis DAD (202 nm). The chromatographic separation of the compounds was achieved using a normal-phase silica (Zorbax RX-Sil column with 4.6 mm ID \times 250 mm, 5 μm particle size), with the corresponding 12.5 mm analytical guard column (both from Agilent Technologies Inc., Palo Alto, CA, USA), at a controlled temperature of 24°C (Jasco CO-2060 Plus, Jasco, Japan). The injection volume ranged between 20 and 100 μl , to get values inside the linearity range of the standard curves, and was eluted with 8% dioxane in n-hexane (v/v) at a flow rate of 1.5 ml/min. Chromatographic data were analysed using a Borwin-PDA Controller Software (JMBS, France). The compounds were identified by retention time comparison with authentic standards. The contents of tocopherols and tocotrienols, as well as the TC, were calculated, in duplicate for each sample, based on the external standard method. Whenever the coefficient of variation between duplicates exceeded 5%, the analyses were repeated. Standard stock solutions of cholesterol in n-hexane (1.0 mg/ml) were formulated and used to prepare standard solutions (0.01, 0.02, 0.05, 0.10, 0.20, 0.40, 0.80, and 1 mg/ml) that were obtained by serial dilutions of the stock solution with n-hexane. A standard stock solution containing 50 $\mu\text{g}/\text{ml}$ of each tocochromanol was prepared. Different dilutions were used for the calibration curves (25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.38 $\mu\text{g}/\text{ml}$).

Lipid Quality Indexes

The peroxidability index (PI) was calculated according to the equation previously proposed by Arakawa and Sagai (1986) as follows:

$$\begin{aligned} & (\% \text{ monoenoic} \times 0.025) + (\% \text{ dienoic} \times 1) \\ & + (\% \text{ trienoic} \times 2) + (\% \text{ tetraenoic} \times 4) \\ & + (\% \text{ pentaenoic} \times 6) + (\% \text{ hexaenoic} \times 8). \end{aligned}$$

The hypocholesterolaemic/hypercholesterolaemic ratio (h/H) was calculated using the equation previously

proposed by Santos-Silva et al. (2002) as follows:

$$\begin{aligned} & [(C18 : 1n - 9 + C18 : 2n - 6 + C18 : 3n - 3 \\ & + C20 : 4n - 6 + C20 : 5n - 3 + C22 : 5n - 3 \\ & + C22 : 6n - 3) / (C14 : 0 + C16 : 0)]. \end{aligned}$$

The indexes of atherogenicity (**AI**) and thrombogenicity (**TI**) were estimated as proposed by Ulbricht and Southgate (1991):

$$\begin{aligned} AI &= (C12 : 0 + 4 \times C14 : 0 + C16 : 0) / \\ & \left[\left(\sum \text{MUFA} + \sum (n - 6) + \sum (n - 3) \right) \right]; \\ TI &= (C14 : 0 + C16 : 0 + C18 : 0) / \\ & \left[\left(0.5 \times \sum \text{MUFA} + 0.5 \times (n - 6) + 3 \right. \right. \\ & \left. \left. \times (n - 3) + (n - 3) / (n - 6) \right) \right]; \end{aligned}$$

The nutritional ratio P/S was calculated as previously established (British Department of Health, 1994) and the n-6/n-3 considering all detected n-6 and n-3 polyunsaturated fatty acid (**PUFA**):

$$\begin{aligned} P/S &= [(18 : 2n - 6) + (18 : 3n - 3) / \\ & (14 : 0 + 16 : 0 + 18 : 0)]; \\ n - 6/n - 3 &= \left[\left(\sum n - 6 \right) / \left(\sum n - 3 \right) \right] \end{aligned}$$

Data and Statistical Analysis

Throughout the results and discussion, the term superiority (expressed as %) was calculated as

$$(\text{maximum value} - \text{minimum value}) / \text{minimum value}.$$

The statistical analysis was accomplished using the MIXED procedure of SAS (SAS Inst., Cary, NC, USA), version 9.3. The model considered three main effects: (1) origin (farmed and wild), (2) meat portion (breast and leg), and (3) sex (male and female), and its interactions. Considering that measurements on different portions from the same animal are not independent observations, portion type was treated as repeated measure within the same animal. Moreover, as none of the variables studied were affected by sex, the model was simplified by removing the sex-fixed factor. Therefore, the second model considered just two main effects, origin (farmed and wild) and meat portion (breast and leg). Least square means were presented and compared using the LSMEANS/PDIFF option when interaction effect was significant ($P < 0.05$). The least square means and standard error of the mean (**SEM**) are presented in tables.

RESULTS AND DISCUSSION

Considering the absence of data on red-legged partridge meat lipid fraction and the absence of data on meat lipid composition in other species belonging to the *Alectoris* genus, the comparison of our results should be extended and include other feathered game species. Recently, our team has published a study on the composition of meat from the common pheasant (*Phasianus colchicus*), which were also produced by the same enterprise, in similar conditions and feeding management (Quaresma et al., 2016). Moreover, *Alectoris rufa* and *P. colchicus* belong to the same family (Phasianidae). Therefore, we will use the data on common pheasant (*P. colchicus*) meat for comparison with red-legged partridge meat.

TL and TC content

The meat TL content (expressed as g/100 g of fresh meat), shown in Table 2, was significantly influenced by the portion ($P < 0.001$), but not by the origin nor by the portion \times origin interaction ($P > 0.05$). The leg meat portion presented a significantly higher content of TL relatively to breast meat portion (averaging 1.47 and 0.86 g/100 g of fresh meat, respectively). Similar results were observed on several other feathered game species reared in Europe, as guineafowl (*Numida meleagris*), common pheasant (*P. colchicus*), Japanese quail (*Coturnix coturnix japonica*), chukar partridge (*Alectoris chukar*), gray partridge (*Perdix perdix*) and also on broiler, rooster, and capon (Vitula et al., 2011; Quaresma et al., 2016, 2017). Greater TL content on leg meat relatively to breast meat portion is probably the consequence of different amounts of intermuscular fat content stored in those portions and the differences in the composition of muscle fiber types. Breast portion is composed by the pectoralis major and minor muscles, whereas leg encloses several different muscles (in both thigh and drumstick), i.e., the area of contact between different muscles is higher in leg than in breast, therefore, the intermuscular fat content stored in leg tends to be higher than in breast, as previously shown in roosters and capons (Tor et al., 2002). Moreover, leg muscles are predominantly constituted by slow-twitch oxidative fibers that use lipid stores as energy reserves, while breast muscles are predominantly composed by fast-twitch glycolytic fibers that use glycogen as major energy reserve (Rosser and George, 1986; Pyörnilä et al., 1998).

The comparison of our results with others obtained from red-legged partridge is not possible since they are unavailable, still it is possible to compare it with the meat TL contents observed on chukar partridge (*A. chukar*) raised under intensive fattening conditions (Jüzl et al., 2012), which reveal to be much fatter (1.8 and 9.8 g/100 g of fresh meat in breast and thigh) than the red-legged partridge presented herein. Such difference could be a consequence of different

Table 2. Total cholesterol (TC), Total lipids (TL), Fatty acid (FA) partial sums, FA ratios, and lipid quality indexes for red-legged partridge meat according to origin and portion.

	Farm			Wild			Statistic		
	Breast	Leg	SEM	Breast	Leg	SEM	Portion	Origin	P*O
TC ¹	40.5 ^b	55.1 ^a	1.030	34.5 ^c	54.2 ^a	1.030	<0.001	0.026	0.009
TL ²	0.90	1.50	0.084	0.82	1.40	0.084	<0.001	0.363	0.706
FA and DMA partial sums ³									
∑SFA	35.5	40.3	0.763	31.3	34.4	0.763	<0.001	<0.001	0.207
∑MUFA	21.7	28.4	2.060	24.7	33.1	2.060	<0.001	0.191	0.439
∑PUFA	32.6	26.5	1.961	31.9	27.9	1.961	0.001	0.895	0.433
∑n-6 PUFA	28.4	24.4	1.793	25.1	24.0	1.793	0.051	0.480	0.249
∑n-3 PUFA	4.24 ^b	2.10 ^c	0.342	6.80 ^a	3.90 ^b	0.342	<0.001	<0.001	0.030
∑n-6 LCFA	15.4	8.27	0.813	12.9	6.66	0.813	<0.001	0.083	0.320
∑n-3 LCFA	3.93 ^b	1.55 ^c	0.255	6.32 ^a	2.48 ^c	0.255	<0.001	0.001	<0.001
DMA	9.71 ^b	4.47 ^c	0.506	11.5 ^a	4.35 ^c	0.506	<0.001	0.193	<0.001
FA and DMA partial sums ²									
∑SFA	0.32	0.63	0.051	0.26	0.49	0.051	<0.001	0.078	0.398
∑MUFA	0.21	0.50	0.063	0.21	0.47	0.063	<0.001	0.880	0.830
∑PUFA	0.28	0.36	0.025	0.26	0.37	0.025	<0.001	0.995	0.352
∑n-6 PUFA	0.24	0.33	0.023	0.21	0.32	0.023	<0.001	0.414	0.387
∑n-3 PUFA	0.04	0.03	0.004	0.06	0.06	0.004	0.049	<0.001	0.318
∑n-6 LCFA	0.13	0.11	0.010	0.11	0.09	0.010	0.005	0.052	0.595
∑n-3 LCFA	0.03	0.02	0.003	0.05	0.03	0.002	<0.001	<0.001	0.138
DMA	0.09	0.06	0.006	0.10	0.06	0.006	<0.001	0.473	0.248
FA ratios and lipid quality indexes									
P/S	0.39	0.44	0.041	0.42	0.58	0.041	0.003	0.163	0.127
n6/n3	6.81 ^b	12.6 ^a	0.574	3.828 ^c	7.22 ^b	0.574	<0.001	<0.001	0.016
h/H	2.32	2.22	0.132	2.85	3.12	0.132	0.418	<0.001	0.093
AI	0.40	0.45	0.023	0.33	0.34	0.023	0.098	0.003	0.188
TI	0.92	0.22	0.043	0.68	0.86	0.043	<0.001	<0.001	0.150

^{a-c}Different superscripts in the same row are associated with significantly different values ($P < 0.05$).

¹Expressed as mg/100 g of fresh meat.

²Expressed as g/100 g of fresh meat.

³Expressed as g/100 g of total FA plus DMA.

feeding management and/or dependent on genetic differences between the two species. Moreover, red-legged partridge presented in this study was raised under similar feeding and management conditions to the common pheasant (Quaresma et al., 2016), and the comparison between the two species revealed that red-legged partridge meat is leaner than pheasant's meat, and the difference observed between breast and leg TL contents is also smaller than was observed on pheasant's meat.

Considering the guidelines of the U.S. Department of Agriculture (2017) for food labeling requirements on meat and poultry, the red-legged partridge meat can be considered as extra lean meat since it contains less than 5 g of total fat, less than 2 g of saturated fat, and less than 95 mg of cholesterol per 100 grams of fresh meat, independently of the meat portion.

The TC content of red-legged partridge meat (depicted in Table 2) was significantly influenced by the statistically significant interaction between portion and origin ($P = 0.009$). The TC content in leg meat portion was significantly higher ($P < 0.05$) than in breast meat portion, independently of the origin. No significant dif-

ferences ($P > 0.05$) were observed between leg meat portions from different origins (averaging 54.7 mg/100 g of fresh meat), but breast meat portion from farmed birds displayed a higher ($P < 0.05$) TC content than was observed on breast meat from their wild counterparts (40.5 vs. 34.5 mg/100 g of fresh meat; a superiority of 17.4%). The higher TC content observed on the leg meat portion comparatively to breast meat portion was also observed on common pheasant (Quaresma et al., 2016) and is probably the consequence of differences in the composition of fibers from breast and leg muscles, as previously explained by others (Chizzolini et al., 1999; Dinh et al., 2011). The red-legged partridge is adapted to perform short and explosive flights and their breast meat (consisting of pectoralis muscles) should be predominantly composed of fast-twitch glycolytic fibers, as previously demonstrated in pheasants and gray partridge (Pyörnilä et al., 1998; Uscebrka et al., 2006; Hofbauer et al., 2010), which possess the largest diameter of all muscle fiber types. The red-legged partridges, as other species from the Phasianidae family, are fast runners, and make use of their legs as the predominant locomotion pattern. The

red-legged partridge leg muscles should be predominantly composed by fast-twitch glycolytic oxidative and slow-twitch oxidative fiber types, as previously demonstrated in other species belonging to the Phasianidae family (Pyörnilä et al., 1998; Uscebrka et al., 2006). Moreover, fast-twitch fibers present a lower concentration of mitochondria than is observed on slow-twitch fibers (Chizzolini et al., 1999).

Muscle fiber type has direct influence in the fiber diameter and mitochondria content, which are both related to the TC content. For the same muscle volume, the smaller the diameter of muscle fibers, the greater is the number of fibers per unit volume. Therefore, higher cell membrane/cytoplasm proportion is associated with higher membrane components as phospholipids and cholesterol. The number of mitochondria per muscle fiber is also an important parameter influencing the amount of membrane components as phospholipids and cholesterol (Chizzolini et al., 1999). Consequently, a higher percentage of slow-twitch oxidative muscle fibers are associated with increased cholesterol content, whereas a higher percentage of glycolytic muscle fibers are associated with lower cholesterol content.

On the other hand, the higher TC content observed on the breast meat portion of farmed relatively to wild partridges may be a consequence of several factors as: (1) oscillations in moisture content, total protein, and lipid contents (Chizzolini et al., 1999); (2) differences in

the birds' feeding management and diet as previously shown in broilers (Crespo and Esteve-Garcia, 2001); (3) differences in muscle fiber composition, since muscle fiber composition is quite variable, even when considering the same muscle within a single species, a consequence of different muscle activity (Chaplin et al., 1997; Lefaucheur, 2010).

FA profile

The FA partial sums, FA ratios, and lipid quality indexes are depicted in Table 2, while the detailed FA and dimethyl acetal (DMA) profile of red-legged partridge' meat (breast and leg portions; expressed as % of total FA plus DMA and g/100 g of fresh meat) is presented for the first time, to the best of our knowledge, in Table 3.

In both meat portions, the saturated fatty acid (SFA) was the prime FA group (33.4% and 37.4% of total FA plus DMA for breast and leg meat portions, respectively). The monounsaturated fatty acid (MUFA) was the second most predominant group in the leg meat portion (30.8% of total FA plus DMA) and least predominant group on breast (23.2% of total FA plus DMA), whereas the PUFA was the second most predominant group in breast (32.3% of total FA plus DMA) and the least predominant group on leg (27.2% of total FA plus DMA).

Table 3. Fatty acid and dimethyl acetal (DMA) profile (expressed as g/100 g of total FA plus DMA) for red-legged partridge meat, according to origin and portion.

	Farm			Wild			Statistic		
	Breast	Leg	SEM	Breast	Leg	SEM	Portion	Origin	P*O
C14:0	0.32	0.70	0.051	0.19	0.44	0.048	<0.001	0.010	0.124
C15:0	0.12	0.20	0.010	0.08	0.12	0.009	<0.001	<0.001	0.096
C16:0	20.4	22.1	0.810	18.1	18.6	0.776	0.155	0.015	0.414
iso-C17:0	0.16 ^{a,b}	0.14 ^b	0.011	0.19 ^a	0.08 ^c	0.010	<0.001	0.268	0.005
C17:0	0.23 ^b	0.33 ^a	0.009	0.18 ^c	0.25 ^b	0.009	<0.001	<0.001	0.009
C18:0	13.9	16.8	0.458	12.2	14.5	0.446	<0.001	0.005	0.393
C20:0	0.21	0.37	0.010	0.17	0.29	0.010	<0.001	<0.001	0.157
C22:0	0.15	0.20	0.015	0.11	0.15	0.014	<0.001	0.034	0.431
C14:1cis-9	0.04	0.09	0.012	0.02	0.08	0.014	0.002	0.498	0.788
C16:1cis-7	0.24	0.43	0.028	0.18	0.39	0.027	<0.001	0.256	0.574
C16:1cis-9	1.29	2.42	0.298	0.75	1.83	0.291	<0.001	0.183	0.294
C18:1cis-9	17.2	23.3	1.715	21.1	28.4	1.683	<0.001	0.074	0.053
C18:1cis-11	2.49 ^b	2.30 ^b	0.139	3.047 ^a	2.13 ^b	0.138	<0.001	0.623	<0.001
C20:1cis-11	0.22	0.450	0.021	0.14	0.32	0.020	<0.001	0.0001	0.141
C18:2n-6	12.9	15.6	1.186	12.2	17.3	1.136	0.002	0.778	0.256
C20:2n-6	0.35	0.260	0.027	0.24	0.15	0.027	<0.001	0.009	0.919
C20:3n-6	0.31	0.15	0.018	0.28	0.15	0.017	<0.001	0.670	0.355
C20:4n-6	13.2	6.63	0.729	11.7	5.92	0.714	<0.001	0.287	0.358
C20:3n-9	0.17 ^a	0.12 ^b	0.012	0.11 ^b	0.08 ^b	0.011	0.004	0.004	0.032
C18:3n-3	0.30 ^b	0.54 ^b	0.206	0.68 ^b	1.42 ^a	0.203	<0.001	0.038	0.015
C20:3n-3	0.09 ^b	0.06 ^c	0.007	0.14 ^a	0.05 ^c	0.007	<0.001	0.080	0.004
C20:5n-3	0.14 ^b	0.27 ^b	0.033	0.22 ^a	0.13 ^b	0.033	0.685	0.512	0.018
C22:5n-3	1.66	0.71	0.119	1.95	0.74	0.116	<0.001	0.346	0.160
C22:6n-3	3.09 ^a	3.95 ^a	0.624	3.84 ^a	1.56 ^b	0.588	0.341	0.349	0.043
C22:4n-6	0.99 ^a	0.60 ^b	0.059	0.40 ^{b,c}	0.32 ^c	0.057	<0.001	<0.001	0.007
C22:5n-6	0.95 ^a	0.50 ^b	0.056	0.37 ^c	0.28 ^c	0.054	<0.001	<0.001	0.002
DMA-16:0	6.81 ^a	3.20 ^b	0.373	8.06 ^a	3.22 ^b	0.364	<0.001	0.235	0.014
DMA-18:0	2.19	0.86	0.155	2.52	0.86	0.152	<0.001	0.459	0.069
DMA-18:1	0.71 ^b	0.23 ^c	0.045	1.02 ^a	0.27 ^c	0.044	<0.001	0.004	<0.001

^{a-c}Different superscripts in the same row are associated with significantly different values ($P < 0.05$).

The total SFA was significantly ($P < 0.001$) influenced by both origin and portion. However, the total MUFA and PUFA were only significantly ($P < 0.05$) influenced by the portion. The farmed specimens presented a significantly ($P < 0.001$) higher SFA contents (more 5.1%) than their wild counterparts. The leg meat portion presented a higher ($P < 0.001$) proportion of SFA and MUFA (4.0% and 7.6% higher, respectively) and a lower proportion of PUFA ($P < 0.001$) than breast meat (5.1% lower). Similar results were observed on pheasant meat, since SFA was also the prime group in both meat portions (34.2% and 44.9% of total FA plus DMA in breast and leg, correspondingly), while MUFA and PUFA were also the second predominant groups in leg (43.1% of total FA plus DMA) and breast (29.5% of total FA plus DMA) meat portions, respectively (Quaresma et al., 2016). However, differences between the red-legged partridge and the common pheasant were observed on major FA groups for both breast and leg meat portions. In breast meat, the red-legged partridge presented a higher percentage of PUFA (2.8%), but a lower percentage of SFA (0.8%) and MUFA (6.5%). Likewise, on leg meat portion, partridge presented a higher percentage of PUFA (16.8%) and lower percentages of SFA (7.5%) and MUFA (12.3%) than was observed on common pheasant (Quaresma et al., 2016).

When considering the FA content expressed as g/100 g of fresh meat, all FA and DMA partial sums were significantly influenced ($P < 0.05$) by portion, whereas n-3 PUFA and n-3 long-chain fatty acid (LCFA) were also significantly influenced ($P < 0.05$) by origin. Breast meat portion presented lower SFA, MUFA, PUFA, and n-6 PUFA, and higher contents of n-3 PUFA, n-6 LCFA, n-3 LCFA, and DMA than leg meat portion. Moreover, meat from farm-raised red-legged partridges tended ($0.05 < P < 0.10$) to present higher contents of SFA and n-6 LCFA.

Regarding PUFA fraction, the n-6 family (n-6 PUFA) was not significantly ($P > 0.05$) influenced neither by the portion nor by the origin and was accountable for 26.8% and 24.2% of total FA plus DMA in breast and leg meat portions, respectively (83.0% and 89.0% of total PUFA, for breast and leg meat, respectively). Although, the n-6 LCFA was significantly ($P < 0.001$) influenced by portion, being higher in breast than leg meat portions (6.7% more).

A statistically significant ($P < 0.05$) interaction between portion and origin was found on the total n-3 family of PUFA (n-3 PUFA), the LCFA of n-3 PUFA (n-3 LCFA), and total DMA, being higher in breast of wild red-legged partridges than in their farmed counterparts. Moreover, no significant differences ($P > 0.05$) were observed between leg meat portion from different origins, exception made for the n-3 PUFA. Comparatively to common pheasant, the red-legged partridge meat presented higher proportion of n-3 PUFA in breast (2.6% more) and leg (2.7% more) meat portions (Quaresma et al., 2016).

The DMA were accountable for 4.4 and 10.7% of total FA plus DMA in leg and breast meat portions, respectively, while in the common pheasant, the DMA was accountable for lower percentages (1.5 and 6.4% of total FA plus DMA in leg and breast meat portion, respectively; Quaresma et al., 2016). DMA are formed from cleavage of alkenyl chains linked to ether bonds in plasmalogenic phospholipids, whenever acid conditions are used to prepare FAME derivatives (Kraft et al., 2008; Alves and Bessa, 2009). Despite the percentage of plasmalogenic phospholipids could fluctuate between different tissues, DMA values are consistent with the results observed on TL contents supporting the idea that breast is richer in phospholipids rather than on triacylglycerols.

Regarding lipid quality indexes and FA ratios, the TI index was simultaneously influenced by both the origin and portion, being higher in farm-raised birds than in wild birds and on leg than on breast meat portion ($P < 0.001$). Besides, the origin has significantly ($P < 0.05$) influenced the hypocholesterolaemic/hypercholesterolaemic (h/H) and the AI indexes, while portion has significantly ($P = 0.003$) influenced the P/S ratio. The n-6/n-3 ratio was associated with a statistically significant interaction ($P = 0.016$) between main effects, revealing that leg meat portion presented higher values than breast portion in birds with the same origin and that meat from farm-raised birds presented higher values than those from their wild counterparts within the same meat portion. Therefore, meat from wild partridge revealed a significantly healthier n-6/n-3, h/H, AI, and TI indexes. Among meat portions, breast meat presented healthier P/S and n-6/n-3 ratios and better TI index.

The evaluation of individual FA revealed 12 statistically significant ($P < 0.05$) interactions between portion and origin on iso-C17:0, C17:0, C18:1 cis-11, C20:3n-9, C18:3n-3, C20:3n-3, C20:5n-3, C22:6n-3, C22:4n-6, C22:5n-6, DMA-16:0, and DMA-18:1. Among farm-raised birds, leg portion showed the highest value of C17:0, while breast portion revealed the highest proportions of C20:3n-9, C22:4n-6, and C22:5n-6. On the other hand, in wild birds higher proportion of C18:3n-3 was observed on leg and higher proportions of iso-C17:0, C18:1 cis-11, C20:3n-3, C20:5n-3, C22:6n-3, DMA-16:0, and DMA-18:1 were observed on breast. Nevertheless, the content of C22:6n-3 did not differ from the values presented by farm-raised red-legged partridge portions, while iso-C17:0 and DMA-16:0 contents did not differ from their counterparts.

Portion has significantly ($P < 0.05$) influenced 16 individual FA, namely C14:0, C15:0, C18:0, C20:0, C22:0, C14:1 cis-9, C16:1 cis-7, C16:1 cis-9, C18:1 cis-9, C20:1 cis-11, C18:2n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:5n-3, and DMA-18:0. Leg portion showed the highest proportions of all SFA and MUFA mentioned above and C18:2n-6. Moreover, all SFA and C20:1 cis-11 were also significantly ($P < 0.05$) influenced by origin, being higher in farm-raised red-legged partridges than their wild counterparts. On

Table 4. Total vitamin E, vitamin E homologues (expressed as $\mu\text{g/g}$ of fresh meat), and the PI in red-legged partridge meat according to origin and portion.

	Farmed		SEM	Wild		SEM	Statistics		
	Breast	Leg		Breast	Leg		Portion	Origin	P * O
Total vitamin E	2.08 ^c	2.25 ^c	0.431	7.79 ^b	9.80 ^a	0.431	<0.001	<0.001	0.002
α -tocopherol	1.38 ^c	1.52 ^c	0.409	7.36 ^b	9.22 ^a	0.409	<0.001	<0.001	0.003
γ -tocopherol	0.11 ^c	0.12 ^c	0.036	0.21 ^b	0.28 ^a	0.036	<0.001	0.020	0.001
α -tocotrienol	0.22	0.029	0.024	—	—	—	0.023	—	—
β -tocopherol	0.03	0.03	0.009	0.05	0.05	0.009	0.717	0.064	0.619
γ -tocotrienol	0.34	0.30	0.027	0.17	0.26	0.027	0.553	0.012	0.099
PI	105.2	63.4	5.524	111.2	66.1	5.524	<0.001	0.582	0.618

^{a-c}Different superscripts in the same row are associated with significantly different values ($P < 0.05$).

the other hand, breast portion presented the highest proportions of C20:2n-6, C20:3n-6, C20:4n-6, C22:5n-3, and DMA-18:0, and none of these FA was significantly ($P > 0.05$) influenced by origin. Differences observed between breast and leg meat portions could be consequence of different proportions of polar and neutral lipid fractions, since polar and neutral lipid fractions enclose a different FA composition (Betti et al., 2009), whereas differences between farmed and wild birds are dependent of the composition of their diet.

Vitamin E Profile

As far as we know, the vitamin E profile of red-legged partridge meat is presented herein for the first time. Data on total vitamin E homologues, tocopherols, and PI are presented in Table 4. It was possible to quantify five tocopherols (α -, β -, and γ -) and two tocotrienols (α - and γ -), while in meat from farm-raised partridges it was possible to quantify only four tocopherols, the same three tocopherols (α -, β -, and γ -) and one tocotrienol (γ -tocotrienol).

Total vitamin E reached its highest content on meat obtained from wild partridges (9.8 and 7.8 $\mu\text{g/g}$ of fresh meat for leg and breast meat portions), which was 4.4 and 3.7 times higher than was observed on leg and breast from farm-raised partridges, respectively. The α -tocopherol was the prime tocopherol among those quantified in red-legged partridge meat, independently of the partridge origin and meat portion, being accountable for 73.7% to 76.8% of total vitamin E in meat from farm-raised partridges and 91.3% to 92.1% of total vitamin E in meat from wild partridges. α -Tocopherol contents were higher in leg than in breast meat portion in both farmed (1.52 vs. 1.37 $\mu\text{g/g}$ of fresh meat) and wild partridges (9.21 vs. 7.35 $\mu\text{g/g}$ of fresh meat). In meat from farm-raised partridges, γ -tocotrienol was the second most predominant tocopherol in both meat portions (0.34 and 0.30 $\mu\text{g/g}$ of fresh meat, being accountable for 18.3% and 15.2% of total vitamin E in breast and leg meat portions, respectively), whereas in meat from wild partridge, the α -tocotrienol was the second most predominant tocopherol independently of the meat portion (0.22 and 0.29 $\mu\text{g/g}$ of fresh meat, responsible for 2.8% and 2.9%

of total vitamin E in breast and leg meat portions, respectively).

A statistically significant interaction ($P < 0.05$) between main effects was observed on total vitamin E, α -tocopherol and γ -tocopherol, significant differences were observed between breast and leg meat portion in wild partridges, but not on farm-raised birds. For the same meat portion, higher values of both tocopherols and total vitamin E were observed in wild than in farm-raised partridges.

The α -tocotrienol was only present in meat from wild partridges and in higher contents in leg than in breast meat (0.29 vs. 0.22 $\mu\text{g/g}$ of fresh meat). The origin has significantly influenced ($P = 0.012$) the meat contents of γ -tocotrienol, and it was observed a statistical tendency on β -tocopherol ($P = 0.064$), where meat from wild birds presented higher contents of β -tocopherol and lower contents of γ -tocotrienol than was observed on farm-raised birds.

Birds and mammals do not possess the necessary biochemical resources to synthesize tocopherols, consequently the tocopherol profile present in red-legged partridge meat and differences observed between farm-raised and wild birds reveal their bioavailability on the diets. Differences between meat portions on their muscle fiber composition, vascularization and metabolism should be responsible for differences observed between portions (Brigelius-Flohé and Traber, 1999; Stocker, 2004; Schneider, 2005).

Among all tocopherols, α -tocopherol is the predominant one independently of the origin or meat portion, such supremacy of α -tocopherol relatively all other tocopherols is dependent of α -tocopherol transfer protein (α -TTP). This is a key-role protein in vitamin E metabolism present in the liver cells, being responsible for vitamin E packing to very low-density lipoproteins, which are major transporters of tocopherols in the bloodstream. α -Tocopherol transfer protein has a considerable higher affinity towards α -tocopherol comparatively to all other tocopherols (Traber and Arai, 1999; Schneider, 2005), such higher affinity is responsible for the predominance of α -tocopherol in meat from both birds and mammals from wild and farm-raised origin (Ponte et al., 2008; Quaresma et al., 2011, 2012, 2013, 2016).

Vitamin E, particularly the α -tocopherol, major lipid-soluble chain-breaking antioxidant present in cell

membranes, is predominantly present in cytoplasmic cell membrane and subcellular membranes, where it stands adjacent to polyunsaturated FA that are vulnerable to free radical attack (Buttriss and Diplock, 1988; Burton and Traber, 1990). α -Tocopherol is able to protect cellular membranes at levels as low as one α -tocopherol per one thousand phospholipids (Dutta and Dutta, 2003). However, the protective effect of α -tocopherol against oxidation and its effects on meat color and on sensory and nutritional characteristics are dependent not only on its concentration, but also on the composition of cell membrane phospholipids, particularly on their unsaturation degree, and the levels of pro-oxidants, as the haeminic iron content (regarded as the prime pro-oxidant molecule in meat; Gatellier et al., 2004).

The PI (calculated by the amount of unsaturated FA and their unsaturation degree) estimates the peroxidation susceptibility of meat's lipid composition. No significant differences ($P = 0.58$) were observed in the PI values from farm-raised and wild partridges, but significant differences ($P < 0.001$) were observed between breast and leg meat portions, being 1.7 times higher in the breast than on leg meat portion. A higher total vitamin E content was observed on meat obtained from wild partridges, independently of the portion, therefore, it is possible to conclude that meat from wild partridges will be less prone to lipid oxidation than their farmed counterparts.

CONCLUSION

The red-legged partridge meat can be considered as extra lean meat, independently of the meat portion and origin. The comparison of farmed and wild birds revealed differences on the FA composition and the tocopherol profile. Meat obtained from the wild specimens presented a healthier lipid quality indexes and improved FA ratios within the meat portion. The absence of significant differences between origins in the PI and the significantly higher total vitamin E content in meat from wild partridges suggest that they are lesser susceptible to lipid peroxidation than farm-raised partridges.

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