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RESEARCH ARTICLE

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Seasonal variations of carcass characteristics, meat quality and nutrition value in Iberian wild red deer

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Abstract

Aim of study: The effects of hunting season (autumn *vs*. winter) on carcass characteristics and meat quality of Iberian wild red deer were assessed.

Area of study: A total of 100 males of wild red deer of Iberian genetic line (*Cervus elaphus*) were hunted on Ciudad Real (south central Spain).

Material and methods: Yields for shoulder (with bone), neck, backbone, loin, tenderloin, leg (with bone), short plate and flank were determined. In addition, samples of *Longissimus thoracis et lumborum* and *Rectus abdominis* muscles were collected. Then, pH_{48}, colour measurements, chemical composition, cooking loss, Warner Bratzler shear blade, fatty acid and amino acid profiles and mineral content were analyzed.

Main results: Deer hunted in autumn (n=50) had higher (p<0.01) yields of shoulder, backbone and short plate and higher contents of intramuscular fat (IMF), cholesterol and K, Fe and Mn but lower (p<0.001) pH_{48} and Na, Mg, Zn and Cu contents than deer hunted in winter (n=50). Shear force tended (p=0.05) to be lower for meat collected in autumn than for meat collected in winter. However, loin yield was 59.2% higher (p<0.001) for winter than for autumn carcasses. Deer hunted in winter had higher α-linoleic acid (p<0.05) and long chain n-3 polyunsaturated (p<0.001) percentages than deer hunted in autumn

Research highlights: Autumn hunting is recommended to obtain carcasses with higher yields of shoulder, backbone and short plate and meat with higher IMF. Conversely, winter hunting is advisable for higher loin yield and for a profile richer in polyunsaturated fatty acids.

Additional key words: *Cervus elaphus*; chemical composition; fatty acids; primal cuts

Abbreviations used: a* (redness); AA (amino acid); b* (yellowness); BW (body weight); C* (chroma); DFD (dark, firm and dry); FA (fatty acid); H° (hue angle); h/H (hypocholesterolemic/hypercholesterolemic ratio); IA (index of atherogenicity); ICP (inductively coupled plasma); IMF (intramuscular fat); IT (index of thrombogenicity); L* (lightness); LTL (*Longissimus thoracis et lumborum*); MUFA (monounsaturated fatty acid); NV (nutritional value); PUFA (polyunsaturated fatty acid); RA (*Rectus abdominis*); SFA (saturated fatty acid); TVA 11t-C18:1 (trans-vaccenic acid).

Authors' contributions: Conceived and designed the experiments: MPS, PdeP, TLC and JML. Performed the experiments and acquired the data: MP and RD. Analyzed the data: MPS and AM. Contributed reagents/materials/analysis tools: AG and LG. Wrote the paper: MPS. All authors revised and approved the final manuscript.

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Introduction

The search for healthier foods and the higher demands in terms of product quality has increased the consumption of meats from alternative animal species raised under natural conditions (Hoffman & Wiklund, 2006; Bureš *et al.*, 2015; Daszkiewicz *et al.*, 2015; Serrano *et al.*, 2019a), such as red deer (Lorenzo *et al.*, 2019; Serrano *et al.*, 2019b). Deer meat is characterized by its high nutrition and sensory quality and by its positive effects on human health resulting from the low contents of intramuscular fat (IMF) and cholesterol and the high contents of protein and minerals (Volpelli *et al.*, 2003; Bureš *et al.*, 2015; Daszkiewicz *et al.*, 2015). This may be one of the reasons why the world trade of game meat has steadily increased to a figure of around two million tonnes annually (Costa *et al.*, 2016).

Most studies about venison include farmed deer which is the common production system in New Zealand, Australia, China and Canada. However, the world production of hunted deer meat seems to be much higher than farm one (Serrano *et al.*, 2019a). Despite being the traditionally method of collecting this meat and reaching nowadays a high production, the scientific information regarding hunted venison is very scarce. Given the importance of carcass composition for meat producers, abattoirs and distributors, recent studies have characterised the carcass from wild red deer (Kudrnáčová *et al.*, 2018; Lorenzo *et al.*, 2019; Maggiolino *et al.*, 2019). In addition, the literature has extensively studied the effects of many factors such as sex (Daszkiewicz *et al.*, 2009; Postolache *et al.*, 2011), muscle type (Zomborszky *et al.*, 1996; Żochowska-Kujawska *et al.*, 2007; Postolache *et al.*, 2011), slaughter age (Dzięciołowski, 1970; Postolache *et al.*, 2011; Lorenzo *et al.*, 2019; Maggiolino *et al.*, 2019) or carcass weight (Żochowska-Kujawska *et al.*, 2007) on meat quality characteristics from wild red deer. Moreover, it has been assessed that hunting period influences growth (Semiadi *et al.*, 1992; Janiszewski *et al.*, 2008), carcass traits (Wiklund *et al.*, 2008) and meat quality (Wiklund *et al.*, 2008, 2010; Stanisz *et al.*, 2019) of cervids. However, the influence of season on primal cut yields, fatty acid (FA) content, amino acid (AA) profile and nutrition value of meat has not been studied in detail for wild red deer. Therefore, the aim of this work was to study the effects of hunting period (autumn *vs.* winter) of wild red deer on carcass characteristics, physicochemical traits and nutrition value of meat.

Material and methods

Experimental design

A total of 100 males of wild red deer of Iberian genetic line (*Cervus elaphus*) with similar body weight (BW) hunted on various game estates (Ciudad Real, south central Spain; supplied by Dibe meat processor who did not revealed name of game estates due to data protection act) to minimise variability caused by inter-regional effects between August 2017 and March 2018 was used in the current study. The average temperature registered in that period was 13.8ºC and relative humidity ranged from 5 to 98%. Deer were distributed in two different groups according to hunting period. The first period was between August and December 2017 (autumn, n=50), while the second period was between January and March 2018 (winter, n=50). Shots entry and exit wounds were in the cranial-thoracic region. All samples were obtained from regular hunting events and, as a result, there was a variation relating to calibres and shooting distance. Animals were exsanguinated, eviscerated and decapitated at the atlanto-occipital junction in the countryside. In each carcass, age was determined by three independent trained wildlife veterinarians (different from the authors of the current paper) who followed guidelines reported by Brown & Chapman (1991), using tooth eruption evaluation, wear patterns and wear score of mandibular molars. In 82% of the carcasses, the estimated age was the same for the three evaluators. In the other carcasses the estimates were slightly different, and the age assigned was the arithmetic mean between the values assessed by the experts as indicated by Lorenzo *et al.* (2019). The mean age of animals was 56 ±7.29 months.

Carcass quality traits

Carcasses were transported under refrigerated conditions to the processing industry (Cárnicas Dibe S.L., Cáceres, Spain), where they were subjected to veterinary examination after evisceration and hide removal. Carcasses were washed with cold water and maintained in a chamber at 0-2ºC for 4 days. Afterwards, as previously indicated by Lorenzo *et al.* (2019), carcasses were weighted and the following carcass measurements were collected: carcass length, leg length, leg width, leg perimeter and the internal depth of chest. After that, cutting stage was performed and shoulder (with bone), neck, backbone, loin, tenderloin, leg (with bone), short plate and flank were weighted, and the results were expressed as the percentage of total carcass weight. From each carcass, samples of *Longissimus thoracis et lumborum* (LTL) from T8 to L6 and *Rectus abdominis* (RA) muscles were collected, vacuum packed and transported to the laboratory (Centro Tecnolóxico da Carne, Ourense, Spain) under refrigerated conditions.

Meat quality traits

Each LTL sample was divided into six steaks. The first three steaks were used to determine pH, colour and

chemical composition. The fourth and fifth steaks were used to determine the cooking loss and the shear force, respectively, whereas the sixth steak was used for the analysis of cholesterol, FA, AA and mineral contents. The external fat was removed from each sample and meat was minced and mixed to produce a homogeneous mixture before samples were submitted to chemical analysis.

Intramuscular pH was recorded at 48 h after hunting on the 9th rib and at 72 h after hunting on the LTL. A digital portable meat pH-meter (Hanna Instruments, Eibar, Spain) with a glass electrode shaped to easily penetrate meat was used for both measurements. At the beginning, the pH meter was calibrated using solutions with pH values of 4 and 7 (Crison, Lainate, Italy) and it was also automatically calibrated for muscle temperature before each measurement as described by De Palo *et al.* (2014). In addition, the incidence of dark, firm and dry (DFD) meats was calculated (pH > 6 at 48 and 72 h *post mortem*).

For colour measurements, LTL and RA samples were allowed to bloom directly in contact with air for 1 h. Objective measures of meat colour (CIE, 1976) including lightness (a greater L* value is indicative of a lighter colour), redness (a greater a* value is indicative of a redder colour) and yellowness (a greater b* value is indicative of a more yellow colour) were determined using a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) with a pulsed xenon arc lamp filtered to illuminate D65 lighting conditions, 0° viewing angle geometry and 8 mm aperture size. Before each series of measurements, the colorimeter was calibrated with a white ceramic tile according to manufacturer recommendations. Three measurements were performed on each sample by rotating the detector system of 90° from the previous on three different points. Then, nine readings per sample were made at each point and averaged for statistical analysis, as described by De Palo *et al.* (2013). Additionally, chroma (C*) and hue angle (H°) were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and H° = arc tan (b*/a*), respectively (Wyszcecki & Stiles, 1982).

Moisture, protein and ashes of LTL were assessed according to the International Organization for Standardization recommended standards (ISO, 1978, 1997, 1998, respectively), while IMF was extracted and quantified according to the American of Oil Chemists´s Society Official Procedure Am 5-04 (AOCS, 2005). Briefly, moisture percentage was calculated by weight loss by the sample maintained in the oven (Memmert UFP 600, Schwabach, Germany) at 105°C until constant weight. Protein content was determined according to Kjeldahl total nitrogen (N) method, multiplying the total N content by 6.25. Sample was subjected to reaction with sulphuric acid (cuprum sulphate was employed as a catalyst) in a digester (Gerhardt Kjeldatherm KB, Bonn, Germany). Organic N was transformed to ammonium sulphate, which was distilled in alkali conditions in a distillation apparatus (Gerhardt Vapodest 50 Carrousel, Bonn, Germany). Ashes percentage was calculated by weight loss experiment by maintaining the sample in a muffle furnace (Carbolite RWF 1200, Hope Valley, England) into a porcelain capsule at 600°C until constant weight. For IMF content determination, samples were subjected to a liquid-solid extraction using petroleum ether 40-60ºC in an extractor apparatus (AnkomHCI Hydrolysis System, Macedon NY, USA) at 90°C during 60 min. The IMF content was obtained based on gravimetric difference.

For determination of total cholesterol of LTL, 2 g of sample was saponified with potassium hydroxide in ethanolic solution, and cholesterol was extracted with n-hexane and separated and identified by normal phase-high performance liquid chromatography (HPLC) technique following the procedure described by Domínguez *et al.* (2018). The HPLC systems used was an Alliance 2695 model (Waters, Milford, USA) equipped with a 996 Photodiode Array Detector (Waters Milford, USA) and 2475 Multi-λ Fluorescence Detector (Waters Milford, USA). Empower 3TM advanced software (Waters, Milford, USA) was used to control system operation and results management. The cholesterol analysis was performed using a normal phase silica column (SunFireTM Prep Silica, 4.6 mm ID × 250 mm, 5 µm particle size, Waters, Milford, MA, USA). The solvent (2% v/v 2-propanol in n-hexane) flow rate was 1 mL/min, the run last for 15 min, and the temperature of the column oven was adjusted at 30 °C. From each standard and sample, 10 µL was injected. The detection of cholesterol was carried out using Photodiode Array detector (PAD) at 208 nm. The total cholesterol content was determined in duplicate for each sample, based on the external standard technique, from a standard curve of peak area *vs*. concentration.

Cooking loss of LTL was calculated as described by Pateiro *et al.* (2013). Briefly, steaks were cooked placing vacuum package bags in a water bath with automatic temperature control (JP Selecta, Precisdg, Barcelona, Spain) until they reached an internal temperature of 70°C, controlled by thermocouples type K (Comark, PK23M, UK), connected to a data logger (Comark Dilligence EVG, N3014, UK). After cooking, samples were cooled in a circulatory water bath set at 18°C for 30 min and the percentage of cooking loss was calculated. The Warner Bratzler shear force was analysed as described by Lorenzo & Carballo (2015). All samples were cut perpendicular to the muscle fibre direction at a crosshead speed of 3.33 mm/min. A texture analyser (TA-XT2, Stable Micro Systems, Godalming, UK) was used. Seven pieces of meat of 1 × 1 × 2.5 cm (height × width × length) were removed parallel to the muscle fibre direction. Samples were completely cut using a Warner Bratzler shear blade with a triangular slot cutting edge (1 mm thickness). Maximum shear force, shown by the higher

peak of the force-time curve, represents the maximum resistance of the sample to the cut.

Fatty acid methyl ester content

For the analysis of FA methyl esters, total fat was extracted from 10 g of ground LTL sample, according to Bligh & Dyer (1959) method. Fifty milligrams of fat were used to determine the FA profile. Total FA were quantified according to Domínguez *et al.* (2015). For the FA transesterification, 4 mL of a sodium methoxide (2%) solution was added to the fat samples, vortexed every 5 min during the 15 min at room temperature, then 4 mL of a H_2SO_4 solution (in methanol at 33%) was added, vortexed for a few seconds and vortexed again before adding 2 mL of distilled water. The organic phase (containing FA methyl esters) was extracted with 2.5 mL of hexane. Separation and quantification of the methyl esters was carried out using a gas chromatograph (GC-Agilent 7890B; Agilent Technologies Spain, S.L., Madrid, Spain), equipped with a flame ionization detector and an automatic sample injector HP 7683, and using a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm internal diameter, 0.2 μm film thickness; Supelco Inc., Bellafonte, PA, USA), following the chromatographic conditions described by Domínguez *et al.* (2015). Individual FA methyl esters were identified by comparing their retention times with those of authenticated standards. Data were used to calculate the total content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA), PUFA/SFA ratio, total content of *n*-6, *n*-3 and long chain *n*-3 PUFA and *n*-6/*n*-3 ratio. Additionally, lipid quality indices were calculated, *i.e.* nutritional value (NV) according to Estévez *et al.* (2004) and hypocholesterolemic/hypercholesterolemic ratio (h/H) according to Santos-Silva *et al.* (2002). Finally, index of atherogenicity (IA) and index of thrombogenicity (IT) were calculated according to Ulbricht & Southgate (1991).

Amino acid profile

Protein hydrolysis, derivatization and identification of hydrolysed AA were carried out following the procedure described by Domínguez *et al.* (2018). Tryptophan determination was not possible because acidic hydrolysis transforms it into ammonium. The HPLC systems and conditions were the same than those used for cholesterol analysis. Empower 3 advanced software (Waters) was used to control the system operation and management of results. Separations were carried out using a Waters AccQ-Tag column (3.9 × 150 mm, with a particle size of 4 μm) with a flow rate of 1.0 mL/min and were performed at 37ºC. Detection was accomplished by fluorescence with excitation at 250 nm and emission at 395 nm.

Mineral content

For mineral determination, ashes previously obtained were dissolved in 10 mL of 1 M HNO_3. The mineral elements (Ca, P, K, Na, Mg, Fe, Mn, Zn and Cu) were quantified by inductively coupled plasma (ICP) optical emission spectrometry, according to the procedure described by Lorenzo & Carballo (2015), using a Thermo-Fisher ICAP 6000 plasma emission spectrometer (Thermo-Fisher, Cambridge, UK), equipped with a radio frequency source of 27.12 MHz, a peristaltic pump, a spraying chamber and a concentric spray nebulizer. The system was totally controlled by ICP software using 99.996% liquid argon plasma gas (Praxair, Madrid, Spain). The final value for the content of each element was calculated as the average of three determinations for each sample.

Statistical analysis

Normal distribution and variance homogeneity were tested before statistical analyses with the Shapiro-Wilk test. All data were normally distributed. A general linear model test was performed to study the effects of hunting period (fixed effect) on carcass and meat quality characteristics and nutrition value traits (dependent variables). Carcass weight was used as covariate for carcass measurements. However, it did not reach significance for any trait considered and, in consequence, it was deleted from the analysis. In all cases, the experimental unit was the sample excised from each individual animal (n=50). Differences were considered significant at $p<0.05$. Values are given as means and standard error of mean. All analyses were carried out with SPSS version 22.0 (2013) (SPSS Inc., NY, USA).

Results

Carcass quality traits

As expected because all deer were hunted with similar BW, the carcass weight was not influenced by season (Table 1). In addition, carcass measurements were not influenced by hunting period, except the chest internal depth that was higher for carcasses hunted in autumn than for carcasses hunted in winter ($p<0.01$). In general, cuts (expressed as percentage of total carcass) varied with hunting period. Thus, yields of shoulder ($p<0.01$), backbone ($p<0.001$) and short plate ($p<0.001$) were higher for carcasses hunted in autumn than for carcasses hunted in winter. However, loin yield was 59.2% higher ($p<0.001$) for winter than for autumn carcasses. On the other hand, hunted period did not affect neck, tenderloin, leg and flank yields.

Table 1. Effects of hunting season on carcass quality traits of Iberian wild red deer.

Item	Hunting season		SEM	*p*-value[1]
	Autumn	Winter		
Carcass weight, kg	35.1	36.7	0.98	NS
Carcass measurements, cm				
Carcass length	86.2	84.5	0.86	NS
Leg length	53.4	54.3	0.43	NS
Leg width	11.6	11.5	0.17	NS
Leg perimeter	61.8	62.3	0.66	NS
Chest depth (internal)	27.7	27.2	0.28	**
Cut yields, % of carcass weight				
Shoulder (with bone)	20.7	20.2	0.11	**
Neck	7.11	6.87	0.117	NS
Backbone	12.4	9.86	0.179	***
Loin	4.81	8.12	0.203	***
Tenderloin	1.31	1.26	0.027	NS
Leg (with bone)	39.0	39.4	0.17	NS
Short plate	5.77	5.27	0.057	***
Flank	8.76	8.84	0.141	NS

[1] NS: not significant ($p>0.10$); **: $p<0.01$; ***: $p<0.001$.

Meat quality traits

Concerning to physicochemical parameters, pH measured on the 9th rib at 48 h *post mortem* was higher for winter than for autumn carcasses (5.89 *vs.* 5.81; $p<0.001$; Table 2). However, the pH measured on the LTL at 72 h *post mortem* did not vary with hunting period. Moreover, the DFD incidence was higher ($p<0.001$) for winter (22%) than for autumn (0%) meats when pH at 48 h *post mortem* was considered, but no differences were detected at 72 h *post mortem* (0 and 2% for autumn and winter, respectively; $p>0.05$).

Hunting period affected in a different way to LTL and RA muscles colour. The LTL muscle from autumn carcasses had higher L* ($p<0.01$), b* ($p<0.01$), C* ($p<0.05$) and Hº ($p<0.01$) than the LTL muscle from winter carcasses. In contrast, values of L* and b* of RA muscle were higher ($p<0.05$) for deer hunted in winter than in autumn.

The average of moisture ranged from 75.6 to 76.0%, IMF from 0.10 to 0.16%, ash from 1.14 to 1.32% and cholesterol from 46.6 to 50.6 mg/100 g. The average protein content was of 22.2% for both groups. Meat chemical composition was affected by hunting period. Thus, loins from deer hunted in autumn had higher contents of IMF ($p<0.01$), ashes ($p<0.001$) and cholesterol ($p<0.01$) and tended ($p=0.06$) to have lower content of moisture than loins from deer hunted in winter. Cooking loss (ranged from 23.8 to 24.3%) was not affected by hunting period, but shear force (ranged from 18.5 to 20.5 N) tended ($p=0.05$) to be higher for winter than for autumn loins.

Fatty acid methyl ester content

The main FA were PUFA (around 44.7%), followed by SFA (around 34.4%) and MUFA (around 19.9%), which showed differences between seasons (Table 3). Meat from deer hunted in autumn had higher contents of SFA ($p<0.01$) and MUFA ($p<0.001$) and lower ($p<0.001$) contents of PUFA than meat from deer hunted in winter. Consequently, winter fat had higher PUFA/SFA ratio and higher contents of *n*-6, *n*-3 and long chain *n*-3 PUFA but lower IT than autumn fat ($p<0.001$). However, differences between seasons did not reach significance for the *n*-6/*n*-3 ratio similarly than for NV, h/H and IA. Moreover, the average *n*-6/*n*-3 ratio was lower than 4 for both seasons.

Regarding SFA, the dominant FA were stearic (C18:0) and palmitic (C16:0) acids with mean values around 16% followed by myristic acid (C14:0) (mean values of 1.5%). These FA did not varied with hunting period. Concerning to MUFA, oleic acid (C18:1*n*-9) was the dominant FA, showing differences between seasons (18.0 *vs*. 10.7% of the total FA for autumn and winter, respectively; $p<0.05$). On the other hand, *trans*-vaccenic acid (TVA; *11t*-C18:1), an important precursor of conjugated linoleic acid, did not differ between collection periods, showing mean values

Table 2. Effects of hunting season on *Longissimus thoracis et lumborum* (LTL) and *Rectus abdominis* (RA) muscle quality traits of Iberian wild red deer.

Item	Hunting season		SEM	*p*-value[1]
	Autumn	Winter		
pH				
At 48 h 9th rib	5.81	5.89	0.012	***
At 72 h LTL	5.65	5.64	0.013	NS
Colour LTL				
Lightness, L*	38.3	36.5	0.36	**
Redness, a*	18.2	17.5	0.21	NS
Yellowness, b*	15.3	13.8	0.26	**
Chroma, C*	23.8	22.3	0.30	*
Hue angle, Hº	0.70	0.66	0.006	**
Colour RA				
L*	36.3	38.2	0.47	*
a*	14.1	14.7	0.27	NS
b*	12.0	13.0	0.25	*
C*	18.6	19.6	0.33	NS
Hº	0.71	0.73	0.008	NS
Chemical composition LTL, g/100 g				
Moisture	75.6	76.0	0.11	0.06
Intramuscular fat	0.16	0.10	0.011	**
Protein	22.2	22.2	0.09	NS
Ash	1.32	1.14	0.016	***
Cholesterol content LTL, mg/100 g	50.6	46.6	0.69	**
Cooking loss LTL, %	24.3	23.8	0.40	NS
Shear force[2] LTL, N	18.5	20.5	0.54	0.05

[1] NS: not significant ($p>0.10$); *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. [2] Warner Bratzler.

of 0.53% of the total FA. Among PUFA, the linoleic acid (C18:2*n*-6) was the main FA representing around 21.4% followed by arachidonic acid (C20:4*n*-6) (around 10.1%) which did not change between collection periods. On the other hand, α-linoleic acid (C18:3*n*-3) and docosapentaenoic acid (C20:5*n*-3) displayed differences between seasons presenting both higher values in meat samples from animals hunted in winter ($p<0.05$).

Amino acid profile

Among the essential AA fraction, the dominant AA was lysine followed by leucine and valine, accounting together about 50% of total essential AAs, whereas methionine presented the lowest values, around 2.5% of total essential AA (Table 4). Regarding the non-essential AA fraction, glutamic acid, aspartic acid and arginine were the main AA observed, representing together around 65% of the total non-essential AA, whereas glycine, serine and proline showed the lowest contents representing together around 24% of total non-essential AA.

In general, essential AA content varied with hunting period. In fact, meat collected in autumn presented higher contents of valine ($p<0.001$), isoleucine ($p<0.05$), phenylalanine ($p<0.001$) and tyrosine ($p<0.001$) than meat collected in winter. For non-essential AA, arginine content was higher ($p<0.001$) in autumn samples than in winter samples, while the content of serine tended ($p=0.08$) to be higher for meat obtained in winter than in autumn.

Mineral content

Potassium was the main mineral (ranging from 247.0 to 318.0 mg/100 g muscle) followed by P (ranging from 220.5 to 224.5 mg/100 g muscle), Na (ranging from 99.6

Table 3. Effects of hunting season on fatty acids profile (g/100 g of total fatty acids) of *Longissimus thoracis et lumborum* from Iberian wild red deer.

Item	Hunting season		SEM	*p*-value[1]
	Autumn	Winter		
C10:0	0.19	0.30	0.009	***
C12:0	0.11	0.10	0.005	0.08
C14:0	1.68	1.30	0.093	NS
C14:1*n-5*	0.49	0.28	0.038	**
C15:0	0.39	0.43	0.020	NS
C16:0	16.0	15.4	0.40	NS
C16:1*n-7*	2.65	1.69	0.120	NS
C17:0	0.44	0.58	0.015	NS
C17:1*n-7*	0.20	0.22	0.007	NS
C18:0	16.6	14.8	0.23	NS
9t-C18:1	0.39	0.14	0.016	***
11t-C18:1	0.61	0.45	0.024	NS
C18:1*n-9*	18.0	10.7	0.45	*
C18:1*n-7*	1.63	2.21	0.060	NS
C18:2*n-6*	18.9	23.8	0.46	NS
C18:2*n-7*	0.19	0.16	0.008	NS
C18:3*n-3*	2.57	4.02	0.155	*
C18:3*n-6*	0.20	0.26	0.008	***
C20:0	0.16	0.14	0.005	NS
C20:1*n-9*	0.01	0.08	0.003	***
C20:2*n-6*	0.11	0.15	0.006	***
C20:3*n-6*	0.76	1.12	0.039	NS
C20:4*n-6*	9.19	10.97	0.267	NS
C20:5*n-3*	1.84	2.80	0.102	*
C22:0	0.07	0.05	0.003	***
C22:2*n-6*	0.10	0.19	0.009	***
C22:5*n-6*	1.72	2.03	0.050	NS
C22:5*n-3*	2.81	4.01	0.112	NS
C22:6*n-3*	0.80	0.63	0.025	NS
SFA[2]	35.6	33.1	0.47	**
MUFA[3]	24.1	15.7	0.53	***
PUFA[4]	39.2	50.1	1.00	***
PUFA/SFA	1.14	1.57	0.046	***
∑*n-6*	31.0	38.5	0.74	***

to 122.2 mg/100 g muscle) and Mg (ranging from 24.1 to 45.7 mg/100 g muscle; Table 5). Hunting period did not affect the LTL contents of Ca and P. However, meat samples collected in autumn presented higher (p<0.001) contents of K, Fe and Mn and lower of Na (p<0.001), Mg (p<0.001), Zn (p=0.06) and Cu (p<0.001) than meat samples collected in winter.

Discussion

Wild deer are typically found in areas with marked seasonal variation in climate and feed supply (Suttie & Webster, 1998). In addition, red deer has a highly seasonal pattern of growth, with maximum accretion of body tissue (muscle and fat) in spring and summer, and minimal

Table 3 (cont.). Effects of hunting season on fatty acids profile (g/100 g of total fatty acids) of Longissimus thoracis et lumborum from Iberian wild red deer.

Item	Hunting season		SEM	*p*-value[1]
	Autumn	Winter		
∑*n-3*	8.02	11.47	0.339	***
Long chain *n-3* PUFA[5]	5.45	7.44	0.211	***
∑*n-6*/∑*n-3*	3.96	3.70	0.106	NS
Nutritional value[6]	0.49	0.50	0.016	NS
h/H[7]	3.14	3.50	0.118	NS
Index of atherogenicity[8]	0.37	0.33	0.014	NS
Index of thrombogenicity[9]	0.63	0.51	0.017	***

[1]NS: not significant (p>0.10);*: p<0.05;**: p<0.01;***: p<0.001. [2]Saturated fatty acids=C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0+C22:0. [3]Monounsaturated fatty acids=C14:1*n*-5+C16:1*n*-7+C17:1*n*-7+9*t*-C18:1+11*t*-C18:1+C18:1*n*-9+C18:1*n*-7+C20:1*n*-9. [4]Polyunsaturated fatty acids=C18:2*n*-6+C18:2*n*-7+C18:3*n*-3+C18:3*n*-6+C20:2*n*-6+C20:3*n*-6+C20:4*n*-6+C20:5*n*-3+C22:2*n*-6+C22:5*n*-6+C22:5*n*-3+C22:6*n*-3. [5]C20:5*n*-3+C22:5*n*-3+C22:6*n*-3. [6]Σ(C12:0+C14:0+C16:0)/Σ(C18:1*n*–9+C18:2*n*–6) as indicated by Estévez *et al*. (2004). [7]Hypocholesterolemic/hypercholesterolemic ratio=[Σ(C18:1*n*–9+C18:1*n*–7+C18:2*n*–6+C18:3*n*–3+C20:3*n*–6+C20:4*n*–6)/Σ(C14:0+C16:0)] as indicated by Santos-Silva *et al*. (2002). [8][C12:0+(4*C14:0)+C16:0]/[(∑MUFA)+(∑PUFA)] as indicated by Ulbricht & Southgate (1991). [9][C14:0+C16:0+C18:0]/[(0.5*∑MUFA)+(0.5**n*–6)+(3**n*–3)+(*n*–3/*n*–6)] as indicated by Ulbricht & Southgate (1991).

accretion, or even loss of body mass, during autumn and winter (Suttie & Webster, 1998). This seasonal variation in protein accretion and catabolism (related to proteolytic enzyme activity) could influence on meat tenderness and other meat quality attributes such as water-holding capacity (Brown & Chapman, 1991). However, and despite being the more common source of deer meat production, available information about the influence of hunting season on carcass and meat quality traits and nutrition value is very scarce for wild red deer. Recently, Stanisz *et al.* (2019) have conducted a pilot study to assess the seasonal variation in the quality of venison from wild fallow deer (*Dama dama*). However and unfortunately, these authors compared meat from fallow deer does hunt-harvested in summer and winter (*vs.* autumn and winter in current study).

Carcass quality traits

In the current study, no differences were observed between carcass weight of deer hunted in autumn and winter. In fact, the season effect cannot be evaluated in the current study because all deer were hunted with similar BW. In contrast, if carcasses had been selected at random, an effect of season on carcass weight would have been expected as previously observed (Wiklund *et al.*, 2008; Gaspar-López *et al.*, 2010; Janiszewski *et al.*, 2010).

The mean weight of the main class meat cuts (shoulder, backbone and leg) constituted as much as 70% of the carcass. The highest mass of meat was obtained from the leg (39.2% as average), followed by shoulder (20.5% as average) and backbone (11.1% as average). The percentage of anatomical joints reported in the current study was very similar to that reported by Maggiolino *et al.* (2019) for wild red deer but differed from the results obtained by Stanisz *et al.* (2015). For example, compared with current results, Stanisz *et al.* (2015) found higher loin (17.7 *vs.* 6.5%) and ribs with flank (16.6 *vs.* 8.8%) yields, but lower percentages of shoulder (16.7 *vs.* 20.5%) and leg (35.2 *vs.* 39.2%) for farmed fallow deer slaughtered with 31-32 months of age. The cause of these differences between studies are unknown but might be due to the disparity in the way of jointing the deer carcasses. In addition, differences between authors could be based on the differences of ages, slaughter method and species compared (average of 56 months of age for wild hunted red deer in the current study *vs*. 31-32 months of age for farmed fallow deer in the study conducted by Stanisz *et al.*, 2015).

Because the economic value depends on the different commercial prize of the different cuts (Sookhareea *et al.*, 2001; Kim *et al.*, 2015; Serrano *et al.* 2019a), to know the carcass composition for wild animals is essential for meat producers, distributors and sellers (Kudrnáčová *et al.*, 2018). Based on cuts expressed as a percentage of total carcass observed in the current study, hunting in winter is recommended when the objective is to obtain a high loin yield, while hunting in autumn is recommended when the objective is to obtain high shoulder, backbone and short plate yields. It is important to mention that differences observed for loin yield between seasons was higher than 59%. However, the literature available about carcass composition and cut incidence of

Table 4. Effects of hunting season on amino acids (AA) profile (mg/100 g of sample) of *Longissimus thoracis et lumborum* muscle from Iberian wild red deer.

Item	Hunting season		SEM	*p*-value[1]
	Autumn	Winter		
Essential AA				
Histidine	790	785	9.78	NS
Threonine	950	938	10.0	NS
Valine	1125	1051	11.0	***
Methionine	227	247	4.12	*
Lysine	1827	1922	21.8	*
Isoleucine	1067	1023	10.7	*
Leucine	1808	1750	18.7	NS
Phenylalanine	957	889	9.27	***
Tyrosine	755	706	7.30	***
Non-essential AA				
Arginine	1843	1638	25.0	***
Aspartic acid	1882	1889	20.8	NS
Serine	789	819	8.45	0.08
Glutamic acid	3128	3166	33.7	NS
Glycine	869	884	8.98	NS
Alanine	1178	1168	14.2	NS
Proline	795	769	7.86	NS
Total AA	19991	19647	197	NS
Essential AA (EAA)	9507	9313	91.37	NS
Non-Essential AA (NEAA)	10485	10334	106.5	NS
EAA/NEAA	0.91	0.90	0.002	NS

[1] NS: not significant (p>0.10); **: p<0.01; ***: p<0.001.

Table 5. Effects of hunting season on mineral composition (mg/100 g of sample except µg/100 g for Mn) of *Longissimus thoracis et lumborum* muscle from Iberian wild red deer.

Item	Hunting season		SEM	*p*-value[1]
	Autumn	Winter		
Ca	6.35	6.49	0.17	NS
P	220.5	224.5	1.74	NS
K	318.0	247.0	4.12	***
Na	99.6	122.2	1.96	***
Mg	24.1	45.7	1.26	***
Fe	3.60	2.91	0.07	***
Mn	22.7	17.5	0.50	***
Zn	1.51	1.61	0.03	0.06
Cu	0.17	0.21	0.004	***

[1] NS: not significant (p>0.10); ***: p<0.001.

wild deer is very scarce. Recently, Martin *et al.* (2019) have compared the lead levels in edible parts (haunch, saddle, area close to wound channel) of red deer hunted with lead or non-lead ammunition. However, these authors did not study any of primal cut yields included 1in current study.

Meat quality traits

Seasonal changes have been reported to have effects on meat quality of deer (Brown & Chapman, 1991; Stanisz *et al.*, 2019) but also for other species such as pork (Dalla Costa *et al.*, 2007; Rodríguez-Sánchez *et al.*, 2009). In the current study, winter samples showed higher values of pH at 48 h *post mortem* than autumn samples. These results agree with those from Wiklund *et al.* (2008) who observed a slightly elevated pH value in meat from deer slaughtered at December (slower growing group) than in meat from deer slaughtered at June (faster growing group). On the other hand, Stanisz *et al.* (2019) also observed an effect of season on pH of fallow deer meat that was higher in the summer compared to the winter season. In addition, values of pH obtained in the current study at 72 h *post mortem* were similar to those obtained by Maggiolino *et al.* (2019) for wild hunted red deer but higher than those obtained in other studies for farmed deer (Żochowska-Kujawska *et al.*, 2007; Purchas *et al.*, 2010; Bureš *et al.*, 2015). These differences, probably, can be due to the fact that the ultimate pH is particularly affected by the slaughtering method. In fact, deer were stunned with a captive-bolt gun before slaughter in some of these studies (Purchas *et al.*, 2010; Bureš *et al.*, 2015), decreasing the stress of deer and, in consequence, modifying the final pH. The great physical effort associated with hunting chase (particularly when deer are chased by dogs as it is most common method of hunting in Spain) reduces glycogen reserves to an insufficient level to ensure a similar pH decline (Daszkiewicz *et al.*, 2015). Therefore, a lack of normal acidification in the muscle during the development of *rigor mortis* is a common phenomenon in game and deer meat, since these species often have low muscle glycogen reserves prior to slaughter and since the available reserves may be depleted if the animals are harvested under stressful conditions (Daszkiewicz *et al.*, 2015). This fact can give rise to an abnormally high ultimate pH values (above 6) resulting in DFD meats that are very common even for farmed deer with incidences up to 50-60% (Daszkiewicz *et al.*, 2015; Serrano *et al.*, 2019b). The incidence is so high that is possible that consumers do not associate the characteristics of this type of meat as a defect but as part of the intrinsic quality of venison meat (Serrano *et al.*, 2019b). In the current study, the incidence of DFD meats was higher for winter (22%) than for autumn (0%) meats when pH at 48 h *post mortem* was considered but no differences were detected at 72 h *post mortem* (0 and 2% for autumn and winter, respectively). In any case, the ultimate pH values obtained in the current study (mean values ranging from 5.64 to 5.65 at 72 h *post mortem*) indicated that the animals were in good physical condition. Therefore, it can be concluded, as reported in literature, that the final pH of deer meat analysed in the current study indicates a high quality of the product despite the stress of the hunting method. Notably that, in general, the pH at 36-48 h *post mortem* has been considered as the ultimate pH for deer meat (Šnirc *et al.*, 2017). However, current results showed that pH continued to decline between 48 (5.81-5.89) and 72 (5.64-5.65) h *post mortem* in the case of deer meat in accordance with Maggiolino *et al.* (2019).

Season influenced on meat colour agree with results obtained previously by Stanisz *et al.* (2019) who observed that the venison obtained in the winter season characterized with higher L* and b* and lower a* compared to meat from the summer season. However, season affected the colour of the LTL and RA muscles differently in current study. In fact, the LTL muscle from autumn carcasses presented higher L*, b*, C* and H^{o} values than the LTL muscle from winter carcasses. In contrast, the RA muscle presented higher values of L* and b* in carcasses hunted in winter. Therefore, it could be interesting to conduct studies about the effects of the interaction between hunting season and muscle from wild deer on meat colour because no similar data are available to compare with current results. According to Volpelli *et al.* (2003), the dark red colour of venison, that is normally attractive to consumers, is characterized by a low L* value (< 40) and by a high a* value (> 21). In the current study, data showed that, although L* was < 40 for LTL and RA muscles, meat showed an average a* value of 17.9 and 14.4 for LTL and RA muscles, respectively. Similar results (L* < 40 and a* > 21) have been previously reported by Serrano *et al.* (2019b) for *Sternocephalicus* and RA muscles from red deer fed on balanced diets and by Maggiolino *et al.* (2019) for LTL and RA muscles from wild hunted red deer.

Values for chemical composition obtained in the current study were similar to those reported in the literature for meat from wild hunted red deer (Maggiolino *et al.*, 2019), for farmed fallow deer (Volpelli *et al.*, 2003) and for red deer feeding on pastures (Purchas *et al.*, 2010). Loins from deer hunted in autumn had higher contents of IMF and cholesterol than loins from deer hunted in winter. These discrepancies might be caused by the differences between these seasons for the amount of food available. As autumn is the season for acorns and other seeds, their high energy content can be transformed in fat than, then, is slowly depleted during winter. This was in agreement with Stanisz *et al.* (2019), who also found a season effect on meat chemical composition. These authors observed that the extractable fat content was higher in winter compared to the summer season in meat from wild fallow

deer. On the basis that the IMF confers juiciness, texture and flavour, especially during heat treatment or cooking, meat from deer hunted in autumn could be more desirable than that from deer hunted in winter. This is the first work in which this type of results are published.

Cooking losses (ranged from 23.8 to 24.3%) were similar to data found by Volpelli *et al.* (2003) for farmed male fallow deer and by Maggiolino *et al.* (2019) for wild hunted red deer but were slightly higher than those reported by Ludwiczak *et al.* (2017) for farmed fallow deer and lower than both those reported by Šnirc *et al.* (2017) for red deer feeding on pastures and by Cawthorn *et al.* (2018) for wild male fallow deer. Differences among authors for the cooking loss values could be due to the method of measurement used. In general, it is difficult to compare results about cooking loss of meat obtained in different studies conducted by different research groups because different authors use different methods to assess water holding capacity and cooking loss which differ, for example, in the temperature and time for cooking. Regarding to treatment effect, in the current study, cooking loss did not vary with hunting season despite the fact that meat samples from deer hunted in autumn had a higher content of IMF.

The finding that shear force tended to be higher for meat obtained in winter than in autumn agrees with results obtained by Wiklund *et al.* (2008). As an average, the shear force observed in the current study was 19.5 N, a value much lower than the 30.2 N observed by Cawthorn *et al.* (2018) for the LTL muscle from male wild fallow deer. However, these values were similar to the 22.1 (stags) and 19.2 (hinds) N observed by Piaskowska *et al.* (2015) for the *Longissimus lumborum* muscle from wild fallow deer and to the 19.2 N observed by Maggiolino *et al.* (2019) as average for the LTL muscle from male wild red deer with different ages at slaughter. The cause of the differences among authors are unknown but variations in muscle tenderness at slaughter and during *post mortem* storage could be the result of various interrelated factors, including pH, amount of connective tissue, IMF content, proteolytic enzyme activity and age of the animal (Cawthorn *et al.*, 2018).

Fatty acid methyl ester content

In the current trial, PUFA were the main FA followed by SFA and MUFA, agree with data reported by Volpelli *et al.* (2003) in male fallow deer and by Lorenzo *et al.* (2019) in male red deer. The main SFA were C18:0 and C16:0 followed by C14:0 agrees with data reported by Quaresma *et al.* (2012) who found that the C18:0 was the main SFA in Iberian red deer meat. Concerning to MUFA, C18:1*n*-9 was the dominant FA agrees with results found in previous studies (Quaresma *et al.*, 2012; Bureš *et al.*, 2015; Daszkiewicz *et al.*, 2015; Lorenzo *et al.*, 2019).

Meat from deer hunted in autumn had higher contents of SFA and MUFA and lower of PUFA than meat from deer hunted in winter. Variations in the forage consumed have a great impact on the FA composition (Wood *et al.*, 2008), so likely the seasonal effect found in the FA profile can be attributed to the change in composition of the deer diet. In fact, the seasonal effect in FA profiles appears even in meat from farmed ruminants due to variations of plant genotypes, harvest times, cutting intervals, fertilization regimes and ensiling techniques applied to forage fed to the animals at different moments of the year (Arvidsson, 2009).

Amino acid profile

In general, the AA profile obtained in this research agrees with this reported for meat from red deer (Lorenzo *et al.*, 2019). Regarding to the influence of hunting season, meat collected in autumn presented higher contents of valine, isoleucine, phenylalanine and tyrosine than meat collected in winter. For non-essential AA, arginine content was higher in autumn samples than in winter samples while the content of serine tended to be higher for winter than for autumn meat. However, no differences were detected for the global content of total, essential and non-essential AA. It is worth highlighting the novelty of these results and, in fact, authors have not found previous studies comparing the AA profile of meat from deer hunted at the most common seasons for hunting (autumn *vs.* winter), as in the current study.

Mineral content

Regarding macro-minerals, K was the main one followed by P, Na and Mg. These results agree with those obtained by Serrano *et al.* (2019b) who found that K was the most abundant mineral in the *Sternocephalicus* and RA muscles of red deer. Although hunting season did not affect the LTL contents of Ca and P, meat samples collected in autumn presented higher contents of K, Fe and Mn and lower of Na, Mg, Zn and Cu than meat samples collected in winter. Previous studies showed a closely related composition of meat minerals with respect to plants from the deer diet (Kudrnáčová *et al.*, 2018). Our results show a similar coherent relationship when comparing meat seasonal trends in mineral compositions in opposition to that of the main four plant sources (acorns, *Quercus* leaves and *Asphodelus* sp.) constituting the diet of deer in the geographical area of Spain were the study was conducted (Estévez *et al.*, 2010). For example: 1) meat K content was higher in autumn than in winter, and it is quite high in acorns (only available in autumn), it was

also higher in *Quercus* leaves in autumn than in winter (opposite trends in the two species of grasses which are consumed mostly in spring and summer); 2) meat Cu content was higher in winter, as it happens with its content in *Quercus* leaves while its content is low in acorns. However, the highest Cu content of all plants is found in *Asphodelus* sp. (a grass), found in mid and late winter, but not in autumn; and 3) finally, meat Zn content was also greater in winter and its content is five times lower in acorns than in other plants, but it is three times higher in *Asphodelus* sp. (a grass not found in winter) than in the other species present in autumn.

It can be concluded that carcass, meat and nutrition value of deer depends on hunting season. In consequence, season is a factor to be taken into account either to decide which quality parameters wished to be obtained. Autumn hunting is recommended to obtain carcasses with high yields of shoulder, backbone and short plate and meat with high IMF content. Conversely, winter hunting is advisable for high loin yield and a more polyunsaturated FA profile.

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