Effect of age on nutritional properties of Iberian wild red deer meat

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ABSTRACT

BACKGROUND: This study assessed the effect of age (L: less than 27 months old, M: from 27 to 42 months old and H: 43 months and older) on fatty acid profile, cholesterol content, amino acid composition and mineral content of 150 Iberian wild red deer meat samples.

RESULTS: Intramuscular fat content increased (P<0.05) with age (0.05 *vs.* 0.12 *vs.* 0.34% for L, M and H groups, respectively) while cholesterol content decreased (P<0.05) as the slaughter age increased (52.78 *vs.* 48.72 *vs.* 45.34 mg/100 g of meat for L, M and H groups, respectively). The slaughter age showed differences among groups for saturated fatty acids showing the highest content in older animals (30.41 *vs.* 34.55 *vs.* 38.21% for L, M and H groups, respectively), whereas younger deer displayed the highest polyunsaturated fatty acid percentages (50.05 *vs.* 45.24 *vs.* 37.55% for L, M and H groups, respectively). The *n*-6/*n*-3 ratio was more favourable (P<0.05) for young and medium ages compared to that from older animals. In contrast, amino acid profile and mineral content were slightly affected by age.

CONCLUSION: As a general conclusion, wild red deer meat could be considered a good alternative to red meats for human consumption.

Keywords: *Cervus elaphus,* amino acids, fatty acids, mineral composition, cholesterol

INTRODUCTION

Meat from animals raised under natural conditions has enjoyed a rise in popularity among consumers in recent years. In this regard, red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) are the most important deer species in Europe, New Zealand, Australia and North America, whereas wapiti (*Cervus canadensis*) and chital deer (*Axis axis*) are the dominant in North America and reindeer (*Rangifer tarandus*) in the Scandinavian countries [1-3].

From a nutritional point of view, meat is known to be an important dietary source of amino acids, proteins and minerals [4]. In this regard, deer meat is a good food commodity for consumers due to several properties, including a low intramuscular fat and cholesterol content and high iron content [5-8]. In addition, this meat presents a high content of unsaturated fatty acids in relation to saturated acids, and is a good source of long-chain *n*-3 polyunsaturated fatty acids (PUFAs) [9-11]. Due to these nutritional properties, deer meat consumption is slowly increasing in several countries based on claims that it is a healthier red meat [6].

In recent years, meat consumers have become increasingly aware of the relationship between health and nutrition, recognizing in the meantime the importance of taste. The concept of meat quality therefore includes taste and technological quality, as well as nutritional value and safety. According to Wood et al. [12], the intramuscular fat (IMF) content of meat affects the fatty acid (FA) profile. In addition, the cholesterol level of meat as well as the fat content and composition are important in relation to consumers' health because they have been associated with obesity,

hypercholesterolaemia and cancer [13]. The incidence of these diseases has increased dramatically in recent years and they are currently the leading causes of death in industrialized countries [14]. In many countries, public health and regulatory agencies have conducted campaigns to raise awareness about the risks of unhealthy diets, and health organizations have recommended lowering the consumption of saturated fatty acids (SFA) and increasing PUFA [15].

The aim of this work was to investigate the effects of slaughter age on the FA profile, amino acid content and macro- and micro- mineral level of wild red deer meat in the Iberian Peninsula. The present study is the first extensive survey of assessment of the nutritional values of wild red deer meat in Spain.

MATERIALS AND METHODS

Animal sampling

For the present study, we used male wild red deer of Iberian genetic line (*Cervus elaphus*) hunted in game estates in Spain between August 2017 and March 2018. Shots entry and exit wounds were in the cranial-thoracic region. All samples were obtained from regular hunting events. There was a variation as regards calibres and shooting distance. Animals were exsanguinated, eviscerated and decapitated at the atlanto-occipital junction in the countryside and carcasses were transported to the processing industry premises (Cárnicas Dibe S.L., Cáceres, Spain) in refrigerated conditions where they were subjected to veterinary examination after evisceration and hide removal. Carcasses were skinned, washed with cold water and maintained in a chamber at 0-2ºC for 4 days.

In each carcass, age was determined by three independent trained persons (wildlife veterinarians) different from the authors of the paper that followed guidelines reported by Brown and Chapman [16], using eruption evaluation, wear patterns and wear score of mandibular molars. In the 81.33% of carcasses, the age estimation were the same for the three evaluators. In the other carcasses, the estimations were slightly different, and the age assigned was the arithmetic mean between the values furnished by the assessors.

On the basis of the estimated age assigned by teeth evaluation, the carcasses were subdivided in three groups (n= 50 animals per group): L (less than 27 months old), M (from 27 to 42 months old) and H (43 months and older). From each carcass, *Longissimus thoracis et lumborum* (LTL) muscle was dissected from T8 to L6, vacuum packed and transported to the laboratory (Centro Tecnolóxico da Carne, Ourense, Spain) in refrigeration conditions for the meat quality analysis. From each sample, the external fat was removed, then was minced and mixed to produce a homogeneous mixture before samples were submitted to chemical analysis procedures.

Intramuscular fat

The IMF content was extracted and quantified according to the AOCS Official Procedure Am 5-04 [17].

Cholesterol analysis

For determination of total cholesterol, 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-HPLC technique following the procedure described by Domínguez et al. [18]. The total cholesterol content was determined, in

duplicate for each sample, using an external standard calibration curve and the results were expressed as mg cholesterol/100 g of meat.

Fatty acid methyl ester analysis

For FA analysis, fat was extracted from 10 g of sample, according to the Bligh and Dyer procedure [19]. Then, fifty milligrams of fat were transesterified according to the procedure described by Domínguez et al. [20]. For the FA transesterification, 4 mL of a sodium methoxide (2%) solution was added to the fat samples, vortexed every 5 min during 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. The FA methyl esters (FAMEs) were separated and quantified using a gas chromatograph (GC-Agilent 7890B; Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector, and using a Supelco SPTM-2560 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness; Supelco Inc., Bellafonte, PA, USA), following the chromatographic conditions described by Domínguez et al. [20]. The FAME composition was expressed in g/100 g of total FAME.

Amino acid analysis

Protein hydrolysis and derivatization and identification of hydrolysed amino acids were carried out following the procedure described by Domínguez et al. [20]. Tryptophan determination was not possible because acidic hydrolysis transforms it into ammonium. The amino acids were analysed with a HPLC (Alliance 2695 model, Waters, Milford, MA) and detected using a scanning fluorescence detector (model 2475, Waters).

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 x 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. The amino acid composition was expressed in mg/100 g of meat.

Mineral analysis

For mineral determination, the previously obtained ashes were dissolved in 10 mL of 1M HNO_3. The mineral elements (Ca, K, Mg, Na, P, Fe, Mn, Zn and Cu) were quantified by inductively coupled plasma - optical emission spectroscopy (ICP-OES), according to the procedure described by Lorenzo et al. [21] 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 each element was calculated as the average of three determinations, previously using an external standard for setting the calibration curve. The results were expressed as mg/100 g of meat.

Statistical analysis

A total of 150 male wild red deer were used in the present study (3 slaughtered age groups x 25 animals per group and replicate x 2 replicates). Normal distribution and variance homogeneity were previously tested (Shapiro-Wilk). Data from FA,

cholesterol, amino acids and minerals content were examined using analysis of variance (ANOVA) with the mixel-model procedure. The parameters mentioned above were included in the model as dependent variables, slaughter age was included as fixed effect, while the hunting period was included as random effect (replicates; where the animals hunted between August and December 2017 were the first replicates, and the animals hunted between January and March 2018 were the second replicates). The pairwise differences between least-square means were evaluated by Duncan's method. Differences were considered significant at $P<0.05$. The values are given as means and standard error (SEM). All statistical analyses were performed using Statistica software (version 8.0.3; Stat Soft Inc. 2007; Tulsa, USA). Correlations between variables ($P<0.05$) were determined using the Pearson's linear correlation coefficient.

RESULTS AND DISCUSSION

IMF and cholesterol content

The effect of slaughter age on the IMF content of Iberian wild red deer is presented in Figure 1. Statistical analysis showed that IMF was significantly ($P<0.05$) affected by slaughter age, showing the highest values in the older animals (0.05 *vs.* 0.12 *vs.* 0.34%, for L, M and H groups, respectively). As expected, the IMF increased as slaughter age increase. A similar trend was observed by others authors in deer [11], foal [22, 23], beef [24], lamb [25] and donkey meat [26]. However, Pinto et al. [27] did not find significant differences in IMF content among fallow deer slaughtered at 3, 6 and 12 months of age. Recorded IMF values were within the range (between 0.24 and 0.30%) observed by Daszkiewicz et al. [2] and Piaskowska et al. [28] for farm-raised fallow deer and hunted

female fallow deer, respectively, but lower than 0.5% found in both studies for meat of hunted males. However, our findings were lower than those reported by other authors who found IMF values ranging from 0.56 to 3.61% in deer meat [7, 9, 10, 29, 30,31].

On the other hand, cholesterol content decreased as the slaughter age increase, showing lower values (P<0.05) in the older animals (52.78 *vs.* 48.72 *vs.*45.34 mg/100 g of meat, for L, M and H groups, respectively; Figure 2). This outcome agrees with data found in Iberian red deer [8] (55.2-55.9 mg/100 g of meat), in free-ranging female elk [32] (50.2 mg/100 g of meat) and in feral Yesosike deer [33] (46.3-58.6 mg/100 g of meat). On the contrary, other authors found higher cholesterol content (ranged from 73.45 to 102 mg/100 g of meat) in deer meat [7, 34, 35]. These differences in the cholesterol content observed in the literature for deer meat could be due to method used for cholesterol determination, type of muscle (LD*, Semitendinosus, Semimembranosus, Psoas major,* etc.), diet, location, sex, animal age, etc. In addition, some authors have found a correlation between cholesterol level in meat and the FA composition in the diet of animals [36].

FA profile

The effect of slaughter age on FA profile of Iberian wild red deer is shown in Table 1. Regarding SFA, the slaughter age showed significant differences among groups, presenting the higher content in older deer (30.41 *vs.* 34.55 *vs.* 38.21%, for L, M and H groups, respectively). On the other hand, PUFA displayed the highest values in younger animals (50.05 *vs.* 45.24 *vs.* 37.55%, for L, M and H groups, respectively). In this regard, variations in IMF level have an influence on the intramuscular FA profile,

mainly on the amount of neutral lipids that tend to be more saturated [37]. This result agrees with data reported by other authors who observed higher monounsaturated fatty acids (MUFA) content in older deer and a higher PUFA amount in younger animals [11, 27, 37]. This fact could be due to higher IMF level in older animals and different relative amounts of triacylglycerol and phospholipids fractions in muscle lipid [6]. In addition, we found a positive correlation between IMF amount and SFA (r= 0.625, P<0.01) and MUFA (r= 0.645, P<0.01) content, whereas a negative correlation between IMF level and PUFA (r= -0.606, P<0.01) contents was observed.

Among SFA, stearic acid (C18:0) was the dominant FA (ranged between 12.73 and 19.13% of the total FA) followed by palmitic acid (C16:0) (varying from 14.40 to 16.01% of the total FA) and myristic acid (C14:0) (ranged from 0.81 to 3.16% of the total FA). A similar trend was reported by Quaresma et al. [8], who noticed that C18:0 was the most abundant SFA in Iberian red deer meat. On the contrary, other authors observed that C16:0 was the main SFA in deer meat [2, 9, 10, 27]. In addition, Volpelli et al. [11] observed a significant (P<0.05) increased on C14:0 content as slaughter age increase (4.6 *vs.* 9.1 mg/100 g of meat for male fallow deer slaughtered at 18 and 30 months, respectively) in agreement with our results. However, Pinto et al. [27] found lower C14:0 amount in older fallow deer (10.63 *vs.* 3.29 *vs.* 8.21%, for animals slaughtered at 3, 6 and 12 months, respectively).

Concerning to MUFA, the most abundant FA was oleic acid (C18:1*n*-9) varying between 11.83 and 14.84% of the total FA, which did not show significant differences among slaughter ages. This result is in agreement with data reported by other authors

who found that C18:1*n*-9 was by far the most abundant of MUFA [2, 7-10]. On the other hand, palmitoleic acid (C16:1*n*-7) displayed significantly (P<0.01) higher values in older animals (1.08 *vs.* 2.06 *vs.* 4.22% of the total FA, for L, M and H groups, respectively). This outcome agrees with data noticed by Volpelli et al. [11] who found significant (P<0.01) higher C16:1*n*-7 in older male fallow deer (5.2 *vs.* 9.4 mg/100 g of meat, for male fallow deer slaughtered at 18 and 30 months, respectively). However, Pinto et al. [27] observed significantly (P<0.01) higher C16:1*n*-7 in male fallow deer slaughtered at 6 months compared to the other ones (11.24 *vs.* 2.60 *vs.* 5.21%, for animals slaughtered at 6, 3 and 12 months, respectively). In addition, *trans* vaccenic acid (TVA) (*11t*-C18:1) an important precursor of conjugated linoleic acid, did not differ among the three groups studied, presenting mean values of 0.53% of the total FA.

Regarding PUFA, the linoleic acid (C18:2*n*-6) was the dominant FA, showing significant (P<0.05) lower values in older animals (23.57 *vs.* 21.06 *vs.* 18.22% of the total FA, for L, M and H groups, respectively). In addition, the amount of the other PUFAs decreased as slaughter age increased (Table 1). This finding could be due to the strong tendency for PUFA concentration to be higher when the IMF content is lower [12] as well as the fact the animals are grazers. In this regard, the highest long chain *n*-3 PUFA were observed in younger animals (7.93 *vs.* 6.63 *vs.* 4.73% of the total FA, P<0.05, for L, M and H groups, respectively). A similar trend was observed by Pinto et al. [27] and Volpelli et al. [11] who found higher PUFA content in younger animals.

On the other hand, it has been reported that ruminant meat has very low *n*-6/*n*-3 ratios, especially if the animals primarily graze, because of the higher levels of linolenic

acid (C18:3*n*-3) found in grass [12]. In our study, the *n*-6/*n*-3 ratio was favourable for young and medium ages (3.54 and 3.71, respectively), whereas for the meat from older animals, the *n*-6/*n*-3 was more than 4.0 (Table 1). However, Volpelli et al. [11] did not find significant differences in the *n*-6/*n*-3 ratios between animals slaughtered at 18 and 30 months, showing mean values of 4.01. Our results agree with the *n*-6/*n*-3 ratios (ranged from 1.21 to 4.75) reported by other authors for deer meat [7-10, 27].

Amino acid profile

Lean deer is an important source of dietary amino acids for human to sustain adequate protein nutrition and health [5]. In fact, meat contains high amounts of protein and balanced proportions of all amino acids relative to human requirements [38]. Table 2 shows the effect of slaughter age on amino acid profile of Iberian wild red deer. All amino acid were not affected by slaughter age, except for lysine, which presented the highest levels in the older animals (1904 *vs.* 1879 *vs.* 1965 mg/100 g of meat, $P<0.05$, for L, M and H groups, respectively).

The three age classes assessed exhibited the following profile: the major amino acid was glutamic acid (around 3200 mg/100 g of meat; around 16% of total amino acids, TAA) followed by lysine and aspartic acid, which showed similar values (around 1900 mg/100 g of meat; around 10% TAA), leucine (around 1750 mg/100 g of meat; around 9% TAA) and arginine (around 1700 mg/100 g of meat; around 8.5% TAA). Arginine was included in the essential amino acids fraction, as it is considered a conditionally essential amino acid [39]. The amino acid profile obtained in this research

agrees with those reported in red deer [40], beef [41], foal [23, 42, 43], lamb [44], donkey [26] and turkey [45] meat.

Regarding the essential amino acids fraction, lysine was the most abundant followed by leucine and arginine, representing together about 52% of total essential amino acids, while, methionine presented the lowest values (ranged from 228 to 250 mg/100 g of meat; representing around 2% of total essential amino acids). On the other hand, glutamic acid, aspartic acid and alanine were the most abundant found in the non-essential fraction, representing together around 66% of the total non-essential amino acids, whereas the lowest values were observed for tyrosine (ranged from 714 to 742 mg/100 g of meat) and proline (between 776 and 798 mg/100 g of meat) representing around 8% of total non-essential amino acids.

Mineral content

The effect of slaughter age on the mineral content of Iberian wild red deer is summarized in Table 3. The most abundant macro-element was potassium (K) (varying from 2.79 to 2.89 g/kg muscle), followed by phosphorous (P) (between 2.17 and 2.35 g/kg muscle) and sodium (Na) (ranged between 1.05 and 1.21 g/kg muscle). Our results depict that the concentrations of all macro-elements did not vary among slaughter ages, except Na that showed significantly (P<0.01) higher values in animals slaughtered at medium age. This outcome agrees with data reported by Grace et al. [46] who observed that K was the main macro mineral, although in this case tissue was not muscle but the liver of grazing red deer. On the contrary, Vengušt and Vengušt [47] found that P was the most abundant macro mineral again in the liver of grazing fallow deer. The mineral

content of deer meat is closely linked to the natural environment as they graze and browse on the grasses, herbs, coniferous species, as well as meadow or crop plants and their meat will thus reflect the mineral composition of their diet [6]. In addition, their concentration varies across muscles and organs, also in relation to the type of physical activity and muscle fibre type composition [48].

Regarding micro-minerals, iron (Fe) was the dominant mineral observed in wild red deer meat, varying from 27.31 to 33.70 mg/kg muscle, without significant differences among slaughter ages (Table 3). This result is in agreement with the range of Fe values (between 26.3 and 34.4 mg/kg muscle) found in roe deer meat [5]. However, these authors noticed that Fe concentration of older female animals was significantly higher than in the younger ones. Our mean Fe concentrations observed in Iberian wild red deer meat were higher than the Fe amounts found in lamb (9.7-17.6 mg/kg) [49] and beef cattle (12-13 mg/kg) [50] meat, and similar to those described in foal (27-31 mg/kg) [42].

On the other hand, our zinc (Zn) values observed in the current study (ranged from 13.62 to 18.32 mg/kg muscle) were slightly lower than those found in roe deer meat (varying between 13.6 and 39.3 mg/kg muscle) [5]. Finally, cooper (Cu) levels varying from 1.93 to 2.11 mg/kg muscle for old and young animals, respectively. Cu is a main essential micro mineral for deer and the liver is the main storage organ playing a key role in its metabolism and reflecting the Cu status of the animal body [6]. Our Cu values were lower than data (ranged from 2.1 and 4.2 mg/kg muscle) found in roe deer meat [5]. Although it was not the aim of this study, the mineral concentrations found in muscles

may vary depending on the stage of growth of antlers (which grow from March until July; not during the period included in this study) as a result of: i) the huge investment for male deer to grow antlers (which can grow at 1 cm/d), and ii) the great importance of some of these minerals for it, such as Ca, P, Zn, Mg and Mn [51].

CONCLUSIONS

The effect of slaughter age had a great impact on intramuscular fat level, fatty acid profile and cholesterol content, whereas amino acid profile and mineral content was slightly affected by slaughter age. The intramuscular fat increases as slaughter age increases, while cholesterol content presented an opposite trend. On the other hand, the fatty acid profile was influenced by slaughter age, presenting the highest polyunsaturated fatty acid content in meat from younger animals. In addition, the meat from older deer showed the highest *n*-6/*n*-3 ratios (above 4). Regarding amino acid and mineral content, the slaughter age had a low impact on both contents. We can conclude that Iberian wild red deer meat can be considered a good source of compounds that are important for human nutrition.

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Table 1. Effect of age on fatty acids profile (g/100 g of total fatty acids) of wild red deer

Fatty acids	Age			SEM	Sig.
	L	M	H		
C14:0	0.81[a]	1.61[b]	3.16[c]	0.130	*
C14:1*n-5*	0.07[a]	0.34[b]	1.06[c]	0.051	*
C15:0	0.30	0.41	0.52	0.016	ns
C16:0	12.73[a]	15.38[b]	19.13[c]	0.380	*
C16:1*n-7*	1.08[a]	2.06[b]	4.22[c]	0.164	**
C17:0	0.49	0.57	0.45	0.013	ns
C18:0	15.57[a]	16.01[a]	14.40[b]	0.177	*
11t-C18:1	0.50	0.51	0.57	0.022	ns
C18:1*n-9*	11.83	13.48	14.84	0.341	ns
C18:1*n-7*	2.01	1.91	2.33	0.058	ns
C18:2*n-6*	23.57[c]	21.06[b]	18.22[a]	0.431	*
C18:3*n-3*	3.73	3.50	2.50	0.132	ns
C20:3*n-6*	1.27	1.00	0.77	0.035	ns
C20:4*n-6*	10.74	10.39	9.06	0.227	ns
C20:5*n-3*	2.98	2.32	1.55	0.086	ns
C22:5*n-6*	2.09	1.97	1.71	0.044	ns
C22:5*n-3*	3.97[c]	3.61[b]	2.62[a]	0.092	*
C22:6*n-3*	0.97	0.69	0.57	0.025	ns
SFA	30.41[a]	34.55[b]	38.21[c]	0.458	*
MUFA	15.28	18.10	22.73	0.444	ns
PUFA	50.05[c]	45.24[b]	37.55[a]	0.898	*
n-6	38.22	34.96	30.16	0.698	ns
n-3	11.66[c]	10.13[b]	7.23[a]	0.292	*
Long chain *n*-3 PUFA[†]	7.93[c]	6.63[b]	4.73[a]	0.182	*
n-6/*n*-3	3.54[a]	3.71[a]	4.33[b]	0.097	*

Ages: L (less than 27 months old), M (from 27 to 42 months old) and H (43 months and older)
SEM: Standard error of the mean
[a-c] Means in the same row with different letters differ significantly ($P<0.05$; test Duncan)
Sig. Significance; ns: not significant; *: P<0.05; **: P<0.01
[†]Long chain *n-3* PUFA (C20:5*n-3*+C22:5*n-3*+C22:6*n-3*)
SFA, saturated fatty acids (C14:0+C15:0+C16:0+C17:0+C18:0)
MUFA, monounsaturated fatty acids (C14:1*n-5*+C16:1*n-7*+C18:1*n-9*+C18:1*n-7*)
PUFA, polyunsaturated fatty acids (C18:2*n-6*+C18:3*n-3*+*11t*-C18:1+C20:3*n-6*+C20:4*n-6*+C20:5*n-3*+C22:5*n-6*+C22:5*n*-3+C22:6*n*-3)

Table 2. Effect of age on amino acids content (expressed as mg/100 g of meat) of wild red deer

Amino acids	Age			SEM	Sig.
	L	M	H		
Essential					
Histidine	802	763	812	7.86	ns
Arginine	1744	1692	1687	15.73	ns
Threonine	953	937	960	7.74	ns
Valine	1088	1065	1130	8.98	ns
Methionine	228	234	250	3.21	ns
Lysine	1904[ab]	1879[a]	1965[b]	16.33	*
Isoleucine	1041	1030	1070	7.82	ns
Leucine	1769	1755	1828	13.82	ns
Phenylalanine	918	901	935	6.66	ns
Non-essential					
Aspartic acid	1894	1877	1961	16.97	ns
Serine	829	795	823	7.01	ns
Glutamic acid	3146	3156	3278	27.91	ns
Glycine	875	875	913	7.29	ns
Alanine	1174	1169	1200	10.42	ns
Proline	776	780	798	5.96	ns
Tyrosine	729	714	742	5.80	ns
Essential	10447	10256	10661	76.53	ns
Non-Essential	9424	9368	9717	73.48	ns
Essential/Non-Essential	1.10	1.10	1.10	0.01	ns

Ages: L (less than 27 months old), M (from 27 to 42 months old) and H (43 months and older)
SEM: Standard error of the mean
[a-b]Means in the same row with different letters differ significantly ($P<0.05$; test Duncan)
Sig. Significance; ns: not significant; *: $P<0.05$

Table 3. Effect of age on mineral content of wild red deer

Minerals	Age			SEM	Sig.
	L	M	H		
Macro-minerals (g/kg)					
Calcium	0.06	0.06	0.06	0.01	ns
Potassium	2.88	2.79	2.89	0.03	ns
Magnesium	0.35	0.38	0.30	0.01	ns
Sodium	1.05[a]	1.21[b]	1.09[a]	0.01	**
Phosphorous	2.35	2.20	2.17	0.01	ns
Micro-minerals (mg/kg)					
Iron	27.31	31.42	33.70	0.58	ns
Manganese	0.22	0.18	0.17	0.01	ns
Zinc	13.62	16.41	18.32	0.28	ns
Copper	2.11	2.02	1.93	0.03	ns

Ages: L (less than 27 months old), M (from 27 to 42 months old) and H (43 months and older)
SEM: Standard error of the mean.
[a-b] Means in the same row with different letters differ significantly (P<0.05; test Duncan).
Sig. Significance; ns: not significant; **: P<0.01

Caption to Figures

Figure 1. Effect of age on intramuscular fat content of Iberian wild red deer. Groups: L (less than 27 months old), M (from 27 to 42 months old) and H (43 months and older). Values expressed as mean±standard error of the mean

Figure 2. Effect of age on cholesterol content of Iberian wild red deer. Groups: L (less than 27 months old), M (from 27 to 42 months old) and H (43 months and older). Values expressed as mean±standard error of the mean

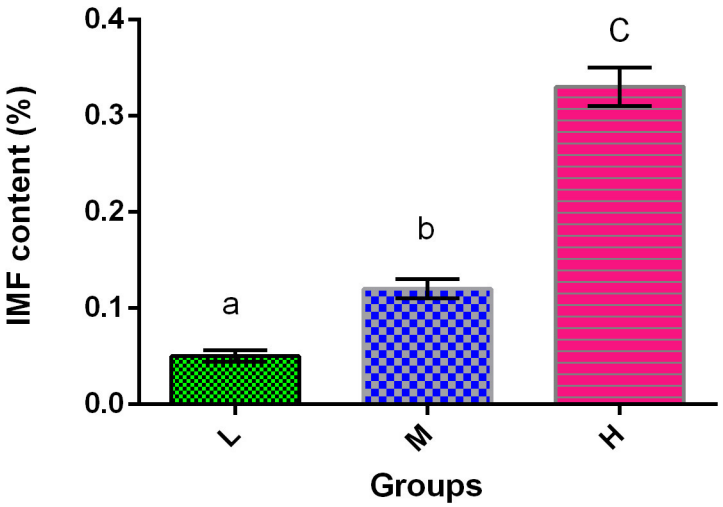

Figure 1.jpg

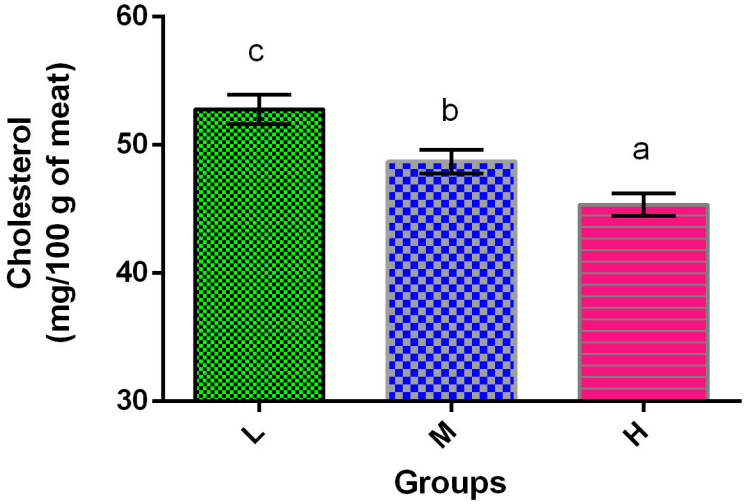

Figure 2.jpg