1 Original Research Article

2 **Lipid and protein quality of Common Pheasant (*Phasianus colchicus*) reared in**
3 **semi-extensive conditions**

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22 **Abstract**

23 Common pheasant (*Phasianus colchicus*) is a species with potential to produce high
24 quality meat. Rearing these birds in semi-extensive regime for other purposes than just
25 for hunting has drawn producers' attention. This study aimed to evaluate the lipid and
26 protein composition of breast and leg meat from male and female pheasant. Meat
27 portions exhibited significant differences between total lipid (1.3 and 3.5 g/100g), total
28 cholesterol contents (0.5 and 0.57mg/g), fatty acids profile, protein content (89 and 82%
29 dry weight) and amino acids profile, not related with sex. The amino acids content
30 tended to be slightly higher in leg, being comparable to farmed pheasants. Lysine was
31 the prevailing essential amino acid (EAA) in both portions, which are also an
32 exceptional arginine source. Pheasant meat is an interesting source of high quality lean
33 protein, being a healthier alternative to other frequently consumed meats.

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37 **1. Introduction**

38 In Europe, game birds can be simplistically grouped into landfowl and waterfowl.
39 Landfowl species pertain to two major orders, galliformes and columbiformes. Several
40 species of both orders are hunted all over the world. In Europe, about 52 million birds
41 belonging to the galliformes and columbiformes orders were shot dead in 2010 (CABS,
42 2010).

43 The common pheasant (*Phasianus colchicus*) is an autochthonous species from central
44 and eastern Asia, from where it was introduced in Europe throughout the expansion of
45 the Roman Empire (Hudec & Cerny, 1977). Currently, the common pheasant is the
46 most abundant, widespread and economically important non-migratory game bird in
47 Europe. It is also the most hunted species, representing 42.1% of the global landfowl
48 species shot in 2010 (CABS, 2010).

49 Nowadays, game meat is a freely available commodity of increasing popularity that
50 represents added diversity to human's diet. The production of game birds in semi-
51 extensive system has been arousing great interest among producers. This type of
52 production system is expected to provide the animals not only a healthy environment,
53 good welfare status, but also an adequate response to their nutritional needs.

54 Similarly to other white meats, pheasant meat has high protein and low fat contents, an
55 interesting proportion of essential and unsaturated fatty acids, and a high content of
56 certain B-group vitamins (Večerek et al., 2005).

57 Besides its nutritional quality, pheasant meat is also recognized for its sensory
58 properties, such as taste, aroma, tenderness and juiciness (Bernacki, 2012; Hofbauer et
59 al., 2010), features that can be affected by the birds' rearing and feeding conditions.

60 The protein content in pheasant meat is known to range between 19.1 and 19.2 g/100 g
61 in thigh muscle and 20.7 - 20.9 g/100 g in breast muscle (Večerek et al., 2005). It
62 contains also higher levels of essential amino acids, as compared with the traditional,
63 currently produced meats. Moreover, the breast muscle of pheasants kept at the farm has
64 revealed a better amino acids profile in comparison with that of wild pheasants
65 (Brudnicki et al., 2012). Regarding the lipid profile, meat from wild animals tends to
66 display a better fatty acid (FA) profile than intensive produced animals (Hoffman &
67 Wiklund, 2006; Quaresma et al., 2011).

68 Thus, the common pheasant can be considered as a species with great potential to
69 produce high quality meat (Marsico & Vonghia, 1991). Despite of that, in most
70 countries, it has been merely raised for hunting purposes.

71 The present study was, therefore, conducted to evaluate the lipid composition (total
72 lipids (TL), total cholesterol (TC), fatty acids (FA) and vitamin E profiles), protein and
73 amino acids profile of common pheasant breast and leg meats. Simultaneously the sex
74 effect on meat nutritional quality was also assessed.

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76 **2. Material and Methods**

77 **2.1. Chemicals and Reagents**

78 Acetone and *n*-Hexane (HPLC grade) were purchased from Merck (Darmstadt,
79 Germany); absolute ethanol (99.8% v/v) from AGA (Lisboa, Portugal); HPLC grade
80 acetonitrile, 1,4-dioxane and N-N-dimethylformamide were from Fluka (Madrid,
81 Spain).

82 High-purity gases, R grade (helium, hydrogen, nitrogen and air) were acquired from Air
83 Liquide (Lisboa, Portugal). It were also acquired: the fatty acids methyl esters (FAME)
84 37 standards mixture from Supelco (Bellefont, PA, USA); 14% boron-trifluoride in
85 methanol, and cholesterol ($\geq 99.0\%$ purity) from Sigma-Aldrich (Steinheim, Germany);
86 tocopherols (α , β , γ and δ) and tocotrienols (α , β , γ and δ) from Calbiochem (La Jolla,
87 California, USA); the internal standard tocol from Matreya Inc. (State College,
88 Pennsylvania, USA); dansyl chloride, L-amino acid standards and glycine (kit
89 containing 21 standards ($\geq 99.0\%$ purity): L-alanine (Ala), L-arginine hydrochloride
90 (Arg), L-asparagine (Asn), L-aspartic acid (Asp), L-glutamic acid (Glu), L-cysteine
91 (Cys), L-cystine, L-glutamine (Gln), L-histidine hydrochloride (His), L-isoleucine (Ile),
92 L-leucine (Leu), L-lysine hydrochloride (Lys), L-methionine (Met), L-phenylalanine
93 (Phe), L-ornithine (Orn), L-4-hydroxyproline (HPro), L-Proline (Pro), L-serine (Ser),
94 L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr) and L-valine (Val)), and the
95 internal standard L-norleucine were from Sigma (St. Louis, MO, USA). Purified water
96 was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).
97 All other chemicals used were of analytical grade.

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99 **2.2 Production system of birds**

100 The common pheasants (*Phasianus colchicus*) used in this study were provided by
101 CAÇABRAVA (Santa Cita, Portugal), a company specialized in game bird rearing for
102 hunting purposes. All rearing steps are performed with the minimum contact to humans
103 to assure birds' braveness.

104 The rearing facilities are original and were designed for game birds and adapted to the
105 specificities of the species. Sheds are composed by 3 different zones: 1) brooder barn;
106 2) night shelter; and 3) rearing pen. Each one is adapted to the growing stage
107 requirements, and was planned to prepare the birds to life in their natural habitat.

108 After hatching, chicks stay in the brooder barn during the first 6 weeks of life. Then
109 they pass to the adaption zone, night shelter, where they remain until 10 - 12 weeks of
110 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.

2.3 Feeding management

Considering the nutritional requirements of the species, in the first 12 weeks of life chicks were fed with a commercial concentrate feeding mixture, especially developed for pheasants and adapted to their age requirements (Provimi, Portugal), which composition is described in Table 1. From the 12nd to the 15th week, the concentrate feeding was progressively replaced by a mixture containing wheat, maize, sorghum, rye, sunflower and oat grains in similar amounts.

2.4 Sampling

Live pheasants, aged 24 - 32 weeks, were collected from the rearing sheds in the preceding morning of the hunting day, placed in cages with water and cereal grains, and then transported to the shooting day place. During the evening period, the cages with the birds were allocated to different zones and opened around 7.00 a.m. The driven shootings were performed in accordance with the national laws on game and hunting. Pheasants were killed by lead shots of 2.5-3 mm diameter, during the morning period.

For this study, pheasants used in a single driven shooting belong to the same flock. The animals were randomly collected among shot birds to provide one male and one female per hunting day. After collection, pheasants were kept in refrigeration (<5° C) until processing (an overall period of 16 - 18 hours).

In the laboratory, birds 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, while 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 portion meat was vacuum packed and frozen at -70 °C until analysis, while the remaining portions were frozen, lyophilized (-60 °C and 2.0 hPa; Edwards High Vacuum International, UK), and maintained desiccated at room temperature, till analysis. This study used shot pheasants ($n=14$, half of each sex), males weighting 952.0 ± 181.5 g and females 708.6 ± 66.1 g (mean $\pm$ standard deviation), collected from seven shooting days in Tomar, Portugal.

2.5 Analytical methods

2.5.1 Total lipids and fatty acids profile

Total lipid (TL) were determined gravimetrically (duplicate values accepted for CV < 5%) from lyophilized meat samples (0.25 g) using a procedure previously described (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 μ L 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 two hours with stirring. FAMES 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.d., 0.25 μ m film thickness, SGE Chromatography Supplies, Austin, TX, USA) was used for FAMES 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 °C/min to 150 °C and held for 1 min, increased again at 1 °C/min to 200 °C, held for 2 min, and finally increased at 3 °C/min to 220 °C. The injector and detector temperatures were maintained at 250 °C. One μ L of sample was injected. Identification of FAMES was achieved by comparison of their retention times with authentic standards.

Tocopherols, tocotrienols and TC determination was performed as previously described (Prates et al., 2006). The chromatographic analysis was performed by HPLC, using an integrated HPLC Jasco System (Jasco, Tokyo, Japan) equipped with a PU-2089 pump,

an AS-2057 automated injector, a FP-2020 fluorescence detector, and a MD-2018 multiwavelength diode array detector (DAD). Tocopherols and tocotrienols were detected/quantified by fluorescence (wavelength excitation at 290 and emission at 330 nm) and cholesterol by UV-Vis DAD (202 nm). The chromatographic separation of the compounds was achieved using a normal-phase silica Zorbax RX-Sil column (4.6 mm ID x 250 mm, 5 µm particle size, with the corresponding 12.5 mm analytical guard column, from Agilent Technologies Inc., Palo Alto, CA, USA), at a controlled temperature of 24 °C (Jasco CO-2060 Plus, Jasco, Tokyo, 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 analyzed using a Borwin-PDA Controller Software (JMBS, France). The compounds were identified by retention time comparison with authentic standards. Tocopherols and tocotrienols, as well as the TC contents, were calculated, in duplicate for each sample, based on the external standard method. Standard stock solutions of cholesterol in *n*-hexane (1.0 mg/mL) was 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 µg/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, 0.38 µg/mL).

2.5.1 Protein and amino acids determination

Protein content was determined by Kjeldahl method (Egli, 2008), and calculated using the conversion factor 6.25. Analyses were performed in duplicate.

The amino acids determination of the pheasant freeze-dried samples was performed after acidic hydrolysis (HCl 6 M, 110 °C, 24h), followed by derivatization with dansyl chloride, as described by Pimentel et al. (2014). The compounds were analyzed by HPLC with fluorescence detection in an HPLC integrated system equipped with an AS-950 automated injector, a PU-980 pump, and a FP-920 fluorescence detector (Jasco, Japan), programmed for excitation at 335 and emission at 514 nm. The chromatographic separation was achieved with a C18 RP Luna column (4.6 x 250 mm, 5 µm) from Phenomenex (Torrance, CA, USA), operating at a controlled temperature of 40 °C (Jasco CO-2060 Plus, Jasco, Japan). The injection volume was 20 µL, eluted with a gradient system consisting in: A) potassium phosphate buffer (0.0185 mol/L) with 4%

N,N- dimethylformamide and 0.1% triethylamine (final pH ~2.5); and B) acetonitrile, at a flow rate of 1.1 mL/min, as follows: 0' 17% B 26' 50% B, 28' 50% B, 40' 90% B. Chromatographic data were analyzed using a Borwin-PDA Controller Software (JMBS, France). The amino acids were identified by comparison of the retention time with authentic standards. Quantification was performed on the basis of the internal standard method using norleucine. A standard stock solution containing 500 µg/mL of each amino acid was prepared. Different dilutions were used for the calibration curves (250, 100, 50, 10, 5 and 1 µg/mL). Results were expressed in mg of amino acid/g of protein.

2.6 Statistical analysis

The first statistical analysis was accomplished using the MIXED procedure of SAS (SAS Inst., Cary, NC, USA), version 9.3. The model considers the effects of sex (male and female), meat portion (breast and leg) and the interaction between sex and meat portion. Since measurements on different portions from the same animal are not independent observations, portion type was treated as repeated measure within the same animal. Least square means were presented and compared using the LSMEANS/PDIFF option when interaction effect was significant ($P < 0.05$). The absence of statistical significant interactions between main effects (sex and meat portion) raised the necessity of a second statistical analysis, following the same model, but without interaction between main effects.

Means were reported as least square means with standard Error of the Mean (SEM).

3. Results and discussion

Data on pheasant meat lipid profile is relatively scarce and, because of that, data available on the USDA National Nutrient Database for Standard Reference was used for comparison (USDA, 2014).

The statistical analysis of data revealed no statistical significant interactions between main effects.

The intramuscular total lipid content (TL; expressed as g/100 g of fresh meat) and fatty acid profile (FA; expressed as relative % of total FA) for breast and leg meat portions are presented in Table 2. Table 3 summarizes the total cholesterol content (TC; expressed as mg/g of fresh meat and the partial sums of different classes of FA, as well as the polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA) and n-6/n-3 ratios.

The TL content of pheasant meat samples was significantly influenced by the meat portion ($P<0.001$), while sex had no significant influence in this variable ($P>0.05$). Breast displayed a significantly lower content of TL than leg meat portion (1.30 vs. 3.46 g/100 g of meat). These differences in the TL contents of breast and leg meat portions are in agreement with results previously published regarding pheasant meat, since breast meat is always the leaner meat portion (Franco & Lorenzo, 2013; Hofbauer et al., 2010; Nuernberg et al., 2011; USDA, 2014). The greater TL content of leg meat compared to breast meat portion is probably the reflex of differences in the amount of intermuscular fat content stored in those portions and the differences in the composition of muscle fibres types. Breast portion is composed by two muscles *pectoralis major* and *minor*, while leg encloses several different muscles (in both thigh and drumstick) that accumulate more fat depots that occur in breast. On the other hand, leg muscles are predominantly constituted by slow-twitch oxidative fibre types that use lipid stores as energy reserves, while breast muscles are predominantly made with fast-twitch glycolytic fibres that use glycogen as major energy reserve. The TL content found on pheasant breast meat portion (1.3 g/100 g of meat) is similar to the TL content previously reported in breast meat of pheasants reared in an extensive system (Franco & Lorenzo, 2013), as well as in farmed and wild Slovakian pheasants (Nuernberg et al., 2011), but considerably above the contents found on breast meat of Austrian pheasants (0.35-0.52 g/100 g of meat) (Hofbauer et al., 2010). The TL content found on pheasant leg meat portion (3.5 g/100 g of meat) is within the range of values previously presented for thigh and leg in farmed pheasants (3.2-4 g/100 g of meat) (Franco & Lorenzo, 2013; Nuernberg et al., 2011), but is considerably above the contents described in leg meat

from wild pheasants (0.8-1.8 g/100 g of meat) (Hofbauer et al., 2010; Nuernberg et al., 2011). Apparently, wild pheasants possess less lipid reserves than farmed ones in breast and leg meat portions probably due to lesser energy intake and more energy expenditure in wild compared to the farmed pheasants.

The intramuscular FA profile of breast and leg meat portions was dominated by SFA in both meat portions (34.2 and 44.9% of total FA, respectively), as presented in Table 3. The palmitic (16:0) and stearic (18:0) acids were the major SFA in both meat portions. Indeed, the total SFA content of breast meat portion is comparable to the total SFA proportion observed in wild (32.2% of total FA) and farmed (32.8-34.9% of total FA) pheasants, while the total SFA content from leg meat portion is above the contents found on both wild (32.0% of total FA) and farmed (30.6-33.9% of total FA) pheasants (Franco & Lorenzo, 2013; Łukasiewicz et al., 2011; Nuernberg et al., 2011).

MUFA was the second most predominant fatty acid group in pheasant meat (29.7 and 43.1% of total FA in breast and leg meat portions, respectively), which are within contents previously reported for both breast (29.3-44.0% of total FA) and leg (36.4-42.6% of total FA) meat portions in farmed pheasant (Franco & Lorenzo, 2013; Łukasiewicz et al., 2011). The oleic acid (18:1*cis*-9) was the predominant MUFA and the major individual FA in pheasant meat.

PUFA was the least predominant FA group in both breast and leg meat portions (29.7 and 10.5% of total FA for breast and leg meat portions, respectively), which is comparable to proportions reported for breast meat in farmed pheasant (23.7-41.0% of total FA), but below the percentage reported for wild pheasant (41.6% of total FA). On the other hand, the PUFA percentage of leg meat portion was quite below the percentage previously reported for both farmed (24.5-42.8% of total FA) and wild (35.5% of total FA) pheasants (Franco & Lorenzo, 2013; Łukasiewicz et al., 2011; Nuernberg et al., 2011).

The portion effect significantly influenced ($P \leq 0.01$) the relative proportion of all FA (exception made for docosanoic acid (22:0), while sex had no significant influence in most of the individual FA ($P > 0.05$), exception made for C16:1 *cis*-7 and C22:5n-6. The

portion has significantly influenced the relative proportion of all FA partial sums ($P < 0.001$), while sex had no significant influence in none of the relative proportion of FA partial sums ($P > 0.05$).

However, the statistical analysis of FA content in meat (g/100 g of fresh meat) revealed slightly different results concerning the portion effect, such differences were observed among total PUFA and n-6 PUFA content (Table 3).

The obtained results regarding the sex effect are, however, in disagreement with results previously presented for farmed pheasant (Łukasiewicz et al., 2011). In the referred study, significant differences ($P < 0.01$) were observed in breast and leg meat portions for total MUFA and total PUFA, while differences for total SFA were limited to leg meat portion ($P < 0.05$). Differences between studies may rely on the birds' age, for example.

Pheasants used in this study were 24 - 32 weeks old, quite older than those used in the Polish one (16 weeks old) (Łukasiewicz et al., 2011). Among galliformes, it is well known that females start the lipid deposition sooner than males. The Polish female pheasants displayed a higher proportion of SFA and MUFA and lower proportion of PUFA, comparatively to male pheasants, what is consistent with the composition of lipid depots. Male galliformes start their lipid deposition later than females, however lipid deposition is a cumulative process and the rate of lipid deposition tends to decrease over time. Therefore, mature pheasants could reach a similar amount of intramuscular fat content, presenting a more uniform fatty acid profile than is observed at younger ages.

The predominance of palmitic and stearic acids among SFA and oleic and linoleic acids among MUFA and PUFA, respectively, is in agreement with the FA profile of pheasant meat from both wild and farmed origins (Franco & Lorenzo, 2013; Łukasiewicz et al., 2011).

Among PUFA, the n-6 family accounted for 90.4 and 96.8% of total PUFA in breast and leg meat portions, respectively. Among n-6 PUFA, the linoleic acid comprised 66.2 and 81.8% of total n-6 PUFA in breast and leg meat portions, respectively. The

arachidonic acid (20:4n-6) comprised for 27.9% and 14.6% of total n-6 PUFA in breast and leg meat portions, respectively.

Docosahexaenoic acid (22:6n-3, DHA) was the predominant n-3 PUFA in both breast (69.4% of total n-3 PUFA) and leg (63.6% of total n-3 PUFA) meat portions, while docosapentaenoic (22:5n-3, DPA) and alpha-linolenic (18:3n3, ALA) acids were the second most predominant n-3 PUFA in breast (17.9% of total n-3 PUFA) and leg meat (36.4% of total n-3 PUFA), respectively. The number of FA belonging to the n-3 PUFA family, and the predominance pattern in different meat portions is in accordance with available data for both breast and leg meat portions, in both wild and farmed pheasants (Franco & Lorenzo, 2013; Łukasiewicz et al., 2011). It is important to highlight that long chain n-3PUFA, and DHA in particular are present in pbreast meat in a considerable higher proportion than their precursor, the alpha-linolenic acid (7.6 times higher). Such result cannot be associated with the diet, since from the 15th week of age until they were released for hunting purposes (24–32th week of age), pheasants were fed with a mixture of grains (wheat, maize, sorghum, rye, sunflower and oat) presented in similar proportions. As it is well known, the predominant fatty acid in these grains is the linoleic acid (C18:2n-6), the alpha-linolenic acid (C18:3n-3) is present in minor amounts, while the long chain n-3 PUFA are absent. Therefore, the results suggest that pheasant possess a considerable capability of elongation and unsaturation of the fatty acids provided by the diet.

The nutritional evaluation of the fat fractions of foods is frequently based on the nutritional indices such as PUFA/SFA and n-6/n-3 ratios (British Department of Health, 1994; Simopoulos, 2002), as well as on the absolute amounts of some health beneficial FA, like 20:5 n-3, 22:5 n-3 and 22:6 n-3 (Givens & Gibbs, 2008; Simopoulos, 2002). The nutritional PUFA/SFA and n-6/n-3 ratios obtained in this study were significantly influenced by the meat portion ($P<0.001$). Breast meat displayed considerably lower PUFA/SFA and n-6/n-3 ratios. In turn, sex had no significant influence on both nutritional ratios ($P>0.05$). The comparison of the results presented herein, with those previously published on pheasant meat revealed that PUFA/SFA ratio is above the

values presented for breast and leg meat portions in farmed and wild pheasant. On the other hand, the n-6/n-3 ratio for breast meat is in between the values presented for farmed pheasant and above the values presented for wild pheasant, while the n-6/n-3 ratio for leg meat is above the values presented for both farmed and wild pheasants (Franco & Lorenzo, 2013; Łukasiewicz et al., 2011).

According to nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFAs (British Department of Health, 1994). The PUFA/SFA ratio obtained is in agreement with nutritional recommendations, above 0.45 in both breast and leg meat portions, to be precise. On the other hand, the n-6/n-3 is regarded as high (averaging 9.7 and 32 for breast and leg meat portions, respectively), making pheasant meat, and the leg meat portion, an excessive source of n-6 PUFAs relatively to n-3 PUFAs (Simopoulos, 2002).

The presence of DMA in pheasant meat is, as far as we know, for the first time reported in this study (Table 2). DMAs are formed from cleavage of alkenyl chains linked to ether bonds in plasmalogenic phospholipids when acid conditions are used to prepare FAME derivatives (Alves & Bessa, 2009; Kraft, Kramer et al., 2008). The differences observed on DMA proportion between meat portions are consistent with PUFA proportion and consequently with the phospholipid content.

Regarding DMA contents, the results revealed that the portion influenced significantly the relative amounts of each DMA and total DMA ($P<0.001$), while sex had no significant effect ($P>0.05$) on single or total DMA (Table 2).

The same three DMA (DMA-16:0, DMA-18:0 and DMA-18:1) were detected both in breast and leg meat portions. Breast displayed a considerably higher content of total DMA than leg (4.4 times when considering the total FA % and 1.6 times per 100 g of fresh meat – Table 3). The presence of DMA is inversely proportional to the amounts of FA involved namely C16, C18 and C18:1.

Concerning total cholesterol content (TC) (Table 3), the meat portion significantly influenced the TC in pheasant meat ($P<0.001$), ranging between 0.50 mg/g in breast and 0.57 mg/g in leg meat portion, what is consistent with contents previously reported

(0.29-0.58 mg/g of meat). The TC content of leg meat portion is below the contents previously reported (0.67-0.80 mg/g of meat) (Franco & Lorenzo, 2013; USDA, 2014). Differences in the TC content between studies may depend on several factors within the same species, namely due to oscillations in moisture content, total protein and lipid contents (Chizzolini et al., 1999). The TC content in meat can also be influenced by the animals' feeding management and diet (Crespo & Esteve-Garcia, 2001). The muscle fibre composition is also quite variable even when considering the same muscle within a single species; such variability is dependent of the maturation degree and production system (Lefaucheur, 2010). The higher TC content of leg meat portion, comparatively to breast meat portion, is a consequence of some differences in muscle fibre composition. Pheasants are adapted to perform short and explosive flights, and their breast meat, predominantly consisting of *pectoralis* muscles, should be predominantly (>70%) composed of fast-twitch glycolytic fibres (Hofbauer et al., 2010; Uscebrka et al., 2006), which possess the largest diameter of all muscle fibre types. Pheasants, as other species from the galliformes order, are fast runners, and make use of their legs as the predominant locomotion pattern. Pheasant leg muscles are predominantly composed by fast-twitch glycolytic oxidative and slow-twitch oxidative fibre types (Uscebrka et al., 2006). Muscle fibre type has direct influence in the fibre diameter and mitochondria content, which are both related to the TC content. For the same muscle volume, the smaller the diameter of muscle fibres, the greater is the number of fibres per unit volume. Therefore, the higher the relative cell membranes/cytoplasm proportion, the higher are the membrane components as phospholipids and cholesterol. The number of mitochondria per muscle fibre is also an important parameter influencing the amount of membrane components as phospholipids and cholesterol (Chizzolini et al., 1999), consequently, higher percentage of oxidative muscle fibres are associated with increased cholesterol content, whereas higher percentage of glycolytic muscle fibres are associated with lower cholesterol content.

Data on vitamin E homologues is presented in Table 4. It was possible to identify and quantify 5 vitamers in pheasant meat, 3 tocopherols (α -, β - and γ -) and 2 tocotrienols (α -

and γ -) in all sampled birds and in both meat portions. Significant differences between meat portions ($P < 0.01$) were detected in α -tocopherol, α -tocotrienol and γ -tocopherol,. Regarding differences between meat portions, leg meat displayed significantly higher content of all three vitamers than breast meat. In turn, sex had no significant influence ($P > 0.05$) in the vitamin E homologues content.

The total vitamin E content in pheasant meat ranged between 4.66 and 6.62 $\mu\text{g/g}$ of meat, for breast and leg meat portions, respectively. α -Tocopherol was the main vitamin E homologue either in breast and in leg meat portions, representing 80.8 and 84.1% of total vitamin E content in breast and leg meat portion, respectively. α -Tocotrienol and γ -tocotrienol were the second and third most predominant vitamin E homologues, in both meat portions.

As far as we know, this is the first time that the vitamin E profile is determined for pheasant meat. Scavenging lipid peroxy radicals is the main role of tocopherols and tocotrienols as antioxidants (Müller et al., 2010). Tocotrienols are also known to help lowering plasma cholesterol levels (Qureshi et al., 1997). Among all vitamers, α -tocopherol is the predominant one in both meat portions, and its amounts are 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 (VLDL), the major transporters in the blood stream. α -TTP has a considerable higher affinity towards α -tocopherol comparing to all other vitamers (Traber & Arai, 1999), consequently α -tocopherol is preferentially distributed throughout body tissues relatively to all others. The predominance of α -tocopherol is a common feature to mammals and birds (Ponte, 2008; Quaresma, *et al.*, 2011). In pheasant meat, it was possible to quantify 5 vitamers, one less than previously published for broiler meat, since δ -tocopherol was also detected. The pheasant breast meat displayed total vitamin E amounts comparable with those of broiler breast meat (Ponte, 2008). In turn, leg portion presented about half of the amounts obtained in breast, either for total vitamin E or α -tocopherol.

As other white meats, analysed pheasant meat contained high protein contents, ranging from 88.3 to 90.1 and from 80.5 to 82.6 g/100 g dry matter, for breast and leg meat portions, respectively. These values are in accordance with the results previously reported (Straková et al., 2011), for the breast portion. Significant differences were detected between portions, but not between sexes.

Pheasant amino acid profile is depicted in Figure 1. The portion had no significant effect ($P>0.05$) in the amino acids contents, exception made for proline ($P=0.003$), which exhibited higher amounts in leg than in breast. In addition, it also appears to exist a statistical trend associated to portion ($P = 0.07$) for glycine and phenylalanine, which presented higher amounts in leg. Regarding sex, significant influence was only perceived for threonine ($P=0.003$), which displayed higher amounts in male than female pheasants. No statistical significant ($P<0.05$) interaction was observed between portion and sex.

Concerning the amino acids profile, it is possible to observe that almost all the essential amino acids (EAA) are present in both portions, except tryptophan. This EAA is destroyed with the acidic conditions used in this determination and, for this reason, it was not quantified.

Lysine, frequently recognized as a protein synthesis-limiting amino acid, was the prevailing EAA in both meat portions, followed by leucine and histidine. The amounts of lysine obtained in this study were higher than those described by Straková et al. (2006), while the amounts of valine and methionine were considerably lower (Straková et al., 2006). The remaining EAA determined were present in amounts under 55 mg/g protein. Within the non-essential amino acids (NEAA), arginine was the most pronounced one.

The sum of EAA was 424.1 and 440.5 mg/g protein in breast and leg portions, respectively, while non-essential amino acids (NEAA) accounted for about 416.8 and 450.7 mg/g protein in breast and leg portions, by this order. Overall, the amino acid profile is in accordance with the results previously presented for pheasant meat (Brudnicki et al., 2012; Straková et al., 2006). Results were similar to those obtained in

farmed pheasant, which generally presents higher amounts of amino acids when compared with the wild ones. Exceptions were observed for lysine and arginine contents, which were considerably higher than those reported in farmed birds, and for glycine, aspartic and glutamic acids which presented much lower amounts than those reported by Straková et al. (2006). Even so, except for valine and methionine, leg amino acids profile presented higher amounts of EAA, compared with the analogous portion from broilers, although the same was not evident for breast portion (Straková et al., 2006).

The amino acid content is important not only because it determines the protein quality, but also because some of them directly influence the taste and flavour, for example. Arginine, for instance, is responsible for the bitter and sweet taste of pheasant meat, while glutamic acid tends to be sour and umami, and alanine is sweet and slightly umami (Bernacki, 2012). The findings reported herein seem to be important features that may direct or indirectly influence the consumption of pheasant meat.

White meats are considered lean and good quality protein sources, when compared with others, such as beef or pork. Its high digestibility, amino acids profile and bioavailability determine them as a high quality protein with superior biologic value (Belitz, 2009).

Nowadays, as the world's population is increasing significantly, it is a great challenge to find new and alternative protein sources to answer to food security concerns in a sustainable manner.

Arginine is a functional amino acid, playing an active role in vascular homeostasis, spermatogenesis, and fetal growth, for example. It is normally synthesized by adults; nevertheless, it is considered a conditional EAA in some special situations, namely when endogenous synthesis is not adequate to meet metabolic needs, what may occur during infant and children's growth, as well as during highly catabolic conditions such as sepsis or burn (van Waardenburg et al., 2007).

The high amounts of arginine found in both meat portions, comparatively to the remaining amino acids, anticipates the possibility of using pheasant meat as an

interesting source of this amino acid throughout the first years of life, in the food diversification period, for example.

Due to the referred above, the promotion of pheasant meat consumption in current food habits would be nutritionally advantageous for consumers, but could also become a new purpose to encourage producers rearing this type of game birds in these semi-extensive conditions.

4 Conclusion

The results of this study showed significant differences between breast and leg meat portions in what concerns its fat fraction. However, few significant differences were associated with the sex effect, which provides pheasant meat a remarkable nutritional uniformity regarding the lipid profile. The protein content also reflected such differences, although in terms of amino acids profile those differences were not so evident.

Pheasant meat can be an exceptional source of high quality lean protein, due to its amino acids profile, with differentiated organoleptic properties, in alternative to other more consumed meats, like poultry, beef, or pork. Nutritional balanced protein sources are desirable and should be fostered in a sustainable manner, in order to allow consumers a wider food choice, but also the availability of the products and the competitiveness of prices. This study anticipates the possibility to promote pheasant rearing for meat production, rather than just for leisure and hunting purposes.

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Figure 1. Amino acid profile (mg/g of protein) of pheasant meat, by portion (breast and leg) and sex.

Legend: EAA – essential amino acids; NEAA – non essential amino acids; His – histidine; Ile – isoleucine; Leu – leucine; Lys – lysine; Val + Met – valine + methionine; Phe – phenylalanine; Thr – threonine; Ala – alanine; Arg – arginine; Asp + Glu Acids – aspartic + glutamic acids; Gly – glycine; Pro – proline; Orn – ornithine; Tyr – tyrosine.

Table 1. Composition of the concentrate feeding provided to pheasant chicks during different growing periods.

Concentrate	I	II	III
Age group	< 3 rd weeks	> 3 th and < 9 th weeks	> 9 th and < 12 th weeks
Crude protein	29.0%	25.0%	21.0%
Crude fiber	3.4%	4.3%	5.8%
Crude fat	3.2%	2.9%	3.4%
Crude ash	7.3%	8.2%	8.5%
Lysine	1.5%	1.3%	0.9%
Methionine	0.6%	0.6%	0.4%
Calcium	1.3%	1.1%	1.2%
Sodium	0.1%	0.2%	0.2%
Phosphorus	0.9%	0.7%	0.7%

* Premix added providing 25 mg/kg of α -tocopherol in all 3 formulations.

Table 2. Total lipids (g/100 g of fresh meat), ~~and~~-fatty acid and dimethylacetals (DMA) profile (g/100 g of total fatty acids and DMA) for pheasant meat according to ~~portion and~~ sex ~~and portion~~.

	Portion		SEM	Sex		SEM	Significance	
	Breast	Leg		Male	Female		Portion	Sex
n	14	14		7	7			
Total lipids	1.30	3.46	0.197	2.20	2.56	0.197	<0.001	0.21
Fatty acids								
C14:0	0.422	0.843	0.0275	0.611	0.654	0.0285	<0.001	0.27
C15:0	0.0985	0.146	0.0101	0.114	0.131	0.0105	0.002	0.24
C16:0	20.9	29.1	0.704	25.1	24.9	0.704	<0.001	0.80
C17:0	0.118	0.188	0.0122	0.152	0.154	0.0121	<0.001	0.91
C18:0	12.4	14.2	0.387	13.3	13.3	0.387	0.003	0.99
C20:0	0.110	0.327	0.0244	0.219	0.218	0.0243	<0.001	0.99
C22:0	0.0964	0.100	0.00841	0.0983	0.0964	0.00874	0.65	0.87
C14:1 <i>cis</i> -9	0.105	0.165	0.0136	0.136	0.134	0.0141	0.005	0.92
C16:1 <i>cis</i> -7	0.175	0.315	0.0105	0.262	0.228	0.0105	<0.001	0.03
C16:1 <i>cis</i> -9	2.81	5.14	0.288	3.94	4.00	0.295	<0.001	0.87
C18:1 <i>cis</i> -9	23.3	34.4	0.654	29.4	28.3	0.655	<0.001	0.26
C18:1 <i>cis</i> -11	3.17	2.82	0.0921	2.89	3.09	0.0925	0.01	0.15
C20:1 <i>cis</i> -11	0.171	0.263	0.0125	0.214	0.219	0.0125	<0.001	0.73
C18:2n-6	17.8	8.26	0.985	12.9	13.2	0.989	<0.001	0.81
C18:3n-3	0.266	0.117	0.0374	0.205	0.179	0.0389	0.009	0.63
C20:2n-6*	0.177	0.0675	0.0115	0.115	0.131	0.0119	<0.001	0.33
C20:3n-6	0.372	0.0616	0.0204	0.217	0.216	0.0202	<0.001	0.98
C20:4n-6	7.48	1.48	0.359	4.41	4.56	0.362	<0.001	0.77
C20:5n-3	0.104	--	0.00654	0.0587	0.0451	0.00680	<0.001	0.16
C22:4n-6	0.700	0.142	0.0388	0.404	0.438	0.0385	<0.001	0.54
C22:5n-6	0.328	0.144	0.0168	0.210	0.262	0.0168	<0.001	0.04
C22:5n-3	0.506	--	0.0404	0.254	0.253	0.0401	<0.001	0.98
C22:6n-3	1.98	0.214	0.131	1.12	1.07	0.132	<0.001	0.81
DMA-C16:0	4.24	0.987	0.185	2.40	2.82	0.185	<0.001	0.12
DMA-C18:0	1.65	0.378	0.0825	0.971	1.05	0.0857	<0.001	0.48
DMA-C18:1	0.485	0.0882	0.0280	0.268	0.306	0.0282	<0.001	0.35

* Co-eluted with C21:0

-- Not detected (below the limit of detection, (<0.12 ppm))

Table 3. Pheasant meat total cholesterol (mg/g of fresh meat), fatty acid partial sums (g/100 g of fresh meat and-as g/100 g of total FA and g/100 g of fresh meat) and nutritional ratios according to portion and sex

	Portion		SEM	Sex		SEM	Significance	
	Breast	Leg		Male	Female		Portion	Sex
n	14	14		7	7			
Total cholesterol	0.368	0.491	0.00974	0.429	0.429	0.00974	<0.001	0.95
Partial sums (expressed as g/100 g of total fatty acids)								
Σ SFA	34.2	44.9	0.856	39.6	39.4	0.856	<0.001	0.87
Σ MUFA	29.7	43.1	0.918	36.9	36.0	0.912	<0.001	0.52
Σ PUFA	29.5	10.4	1.21	19.7	20.2	1.22	<0.001	0.78
Σ DMA	6.38	1.45	0.277	3.65	4.19	0.275	<0.001	0.18
Σ n-6 PUFA	26.7	10.1	1.13	18.1	18.7	1.12	<0.001	0.73
Σ n-3 PUFA	2.86	0.330	0.161	1.64	1.55	0.161	<0.001	0.71
Partial sums (expressed as g/100 g of fresh meat)								
Σ SFA	0.446	1.57	0.105	0.928	1.08	0.105	<0.001	0.30
Σ MUFA	0.389	1.49	0.0842	0.864	1.01	0.0844	<0.001	0.22
Σ PUFA	0.383	0.351	0.0377	0.348	0.385	0.0376	0.55	0.49
Σ DMA	0.0815	0.0490	0.00519	0.0604	0.0701	0.00524	<0.001	0.20
Σ n-6 PUFA	0.346	0.3339	0.0359	0.322	0.362	0.0357	0.89	0.44
Σ n-3 PUFA	0.0365	0.0112	0.00273	0.0251	0.0226	0.00273	<0.001	0.52
Ratios								
PUFA/SFA	0.537	0.199	0.0298	0.363	0.372	0.0298	<0.001	0.83
n-6/n-3	9.84	32.3	1.85	19.5	22.6	1.90	<0.001	0.25

Table 4. Vitamin E homologues ($\mu\text{g/g}$ of fresh meat) according to portion and sex .

	Portion		SEM	sex		SEM	Significance	
	Breast	Leg		Male	Female		Portion	Sex
	n							
	14	14		7	7			
α -tocopherol	3.77	5.57	0.372	4.98	4.36	0.492	0.001	0.38
α -tocotrienol	0.296	0.395	0.0492	0.277	0.413	0.0646	0.01	0.15
β -tocopherol	0.0909	0.0924	0.00559	0.0881	0.0952	0.00765	0.59	0.52
γ -tocopherol	0.289	0.348	0.0312	0.301	0.335	0.0312	<0.001	0.58
γ -tocotrienol	0.216	0.217	0.0166	0.204	0.229	0.0212	0.96	0.41