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Fatty acid signatures of duck breast meat from three varieties

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ABSTRACT

Duck meat is increasingly valued for its distinctive sensory and nutritional properties. Breast muscle samples (pectoralis major and minor) were collected from three duck groups differing in genetic background and rearing system: intensively reared Pekin ducks (PD; n = 47), semi-extensively reared mallards for hunting purposes (MDH; n = 14), and wild mallards (WMD; n = 20), all with balanced sex representation.

This study assessed the origin effect on fatty acid (FA) composition. WMD showed the most favorable lipid profile, with higher polyunsaturated fatty acids (PUFA), especially n-3 PUFA and n-3 long-chain PUFA (LCPUFA), and superior lipid quality indices. MDH exhibited profiles dominated by saturated and monounsaturated FAs, reflecting enhanced de novo lipogenesis and lower nutritional quality, while PD displayed intermediate characteristics with generally balanced FAs but some nutritional limitations.

FA variability was probably driven by dietary inputs and endogenous metabolic pathways, including differences in precursor availability and enzymatic activity in elongation and desaturation. Differences in muscle fiber composition and lipid partitioning between neutral and polar lipid fractions may also contribute to variations in FA profiles. Despite the presence of beneficial FAs such as eicosapentaenoic and docosapentaenoic acids (EPA and DHA, respectively), their absolute levels were low, indicating duck meat is not a major source of these nutrients. Nonetheless, WMD FA composition may positively contribute to dietary lipid intake within a balanced diet.

Canonical discriminant analysis demonstrated that intramuscular FA profiles effectively differentiate duck meat varieties, achieving complete classification accuracy. These results highlight the utility of FA profiling as a reliable tool for authentication and differentiation in duck meat varieties.

Introduction

Duck meat has gained increasing attention worldwide as a high-value product due to its distinctive sensory attributes and nutritional profile. According to FAO, 4.22 billion ducks were slaughtered worldwide in 2024, yielding 7.33 million tons of duck meat (FAOSTAT, 2025). On the other hand, during a single hunting season in Europe, approximately 6.6 million waterfowl specimens belonging to the Anatidae family were harvested, including 4.5 million Mallard ducks (*Anas platyrhynchos*) (Hirschfeld and Heyd, 2005).

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There is considerable diversity among duck breeds, each characterized by distinct productive traits. In general terms, they can be classified into breeds specialized in meat production, egg production, or both.

Currently, duck production is based mainly on two species: the Pekin duck (*Anas platyrhynchos domesticus*), from which most commercial breeds are derived, and the Muscovy duck (*Cairina moschata*). Moreover, interspecific hybrids such as the Mulard, obtained by crossing Muscovy drakes with Pekin females, are exploited for niche markets such as foie gras production (Su, 2022). Duck production is distinguished by the species' adaptability, modest housing requirements, sustained productivity, rapid growth, easy management, and relative resistance to disease (Jalaludeen et al., 2022). Such robust production traits enable ducks to thrive under diverse production systems, ranging from confinement with intensive management to flexible extensive rearing practices. Moreover, ducks are particularly well-suited for integration into mixed farming systems involving fish, rice, rice–fish, and rice–fish–azolla combinations (Jalaludeen et al., 2022).

Consumer interest in duck meat has grown recently, driven by its distinctive flavor and nutritional profile, and it is valued as a unique alternative to traditionally consumed meats (Baéza and Huang, 2022; Padhi et al., 2024). Concerning meat quality attributes, particular concern is directed toward the lipid fraction, both in terms of its content and composition. Over the past decades, the importance of fat levels and fatty acids (FA) profiles has been extensively studied, leading to the establishment of nutritional guidelines (FAO, 1998, 2008, 2010; EFSA, 2016). Fatty acids are natural components of the diet that can perform vital metabolic, structural, and functional roles by providing energy, forming cell membrane structures, and generating signaling molecules that modulate cell activity (Calder and Burdge, 2012). In this regard, age, diet, genetics, production practices, and feeding management are important variables influencing the FA profile and total fat content (Chartrin et al., 2005; Witak, 2008; Onk et al., 2019; Fan et al., 2020; Zhang et al., 2022; Wasilewski et al., 2023).

The primary objective of this study was to characterize the FA profile and nutritional value of duck meat across three contrasting origins: (i) intensive indoor rearing of Pekin ducks (PD), representing a high management condition; (ii) semi-extensive reared mallard ducks receiving partial feed supplementation (MDH), representing an intermediate level of human intervention; and (iii) wild mallard ducks (WMD, reflecting natural conditions without human control. A secondary objective was to evaluate the potential of the FA profile as a discriminative tool for distinguishing among these three meat duck varieties.

Materials and methods

Sampling

Breast meat samples, comprising the *pectoralis major* and *pectoralis minor* muscles, were exclusively used in this study. The study comprises three different duck groups, differing in both genetic background and the feeding management employed.

Samples from Pekin ducks (*Anas platyrhynchos domesticus;* PD) reared under intensive farming conditions, were obtained from parent ducks from Cherry Valley Farms Ltd (n = 25) and Grimaud Frères (n = 22) strains, with balanced sex representation. The rearing conditions, feed composition, and genetic background of these strains were previously described in detail (Quaresma et al., 2025). Mallard ducks (*Anas platyrhynchos*), also with balanced sex representation, were obtained from two distinct sources: 1) individuals reared under semi-extensive conditions for hunting purposes (MDH; n = 14), as previously comprehensively described by Quaresma et al. (2024); and 2) wild individuals (WMD; n = 20), legally harvested in accordance with the Portuguese hunting legislation by licensed hunters and subsequently provided to the research team.

Chemicals

All reagents used in this study were of appropriate analytical grade. Pellet potassium hydroxide (KOH), methanol, and sulfuric acid (H_2SO_4) were of pro analysis quality, whereas n-hexane was suitable for gas chromatography. All chemicals were obtained from Merck Biosciences (Darmstadt, Germany). A standard mixture of 37 fatty acid methyl esters (FAME) was purchased from Supelco Inc. (Bellefonte, PA, USA), while the all-cis-7,10,13,16,19-docosapentaenoic acid (C22:5n-3), all-cis-4,7,10,13,16-docosapentaenoic acid (C22:5n-6), cis-7,10,13,16-docosatetraenoic acid (C22:4n-6) and butylated hydroxytoluene (BHT, ≥99%) were all obtained from Sigma-Aldrich (St. Louis, MO, USA).

Fatty acids determination and identification

The FA profile of breast meat samples was analyzed according to the method of O'Fallon et al. (2007), with slight modifications. In brief, 1 g of fresh sample was transferred into a 16 × 125 mm screw-cap Pyrex tube. An internal standard solution (0.5 mL of C13:0 at 1.0 mg/mL in methanol), along with 0.7 mL of 10 M KOH and 5.3 mL of methanol, was then added. The mixture was incubated in a water bath at 55°C for 90 min, with manual shaking for approximately 5 s every 15 min.

Following incubation, the tubes were cooled under running tap water, after which 0.58 mL of 12 M H_2SO_4 was added. The contents were gently mixed by inversion and subjected to a second incubation under identical conditions.

Upon completion of FAME synthesis, the tubes were again cooled in tap water. Next, 3 mL of n-hexane containing 0.02% butylated hydroxytoluene (BHT) was added, and the samples were vortex-mixed for 5 min using a multi-tube vortex (Heidolph, Nuremberg, Germany). The mixtures were then centrifuged for 5 min using a benchtop centrifuge. The upper hexane layer, containing the FAME, was carefully collected, transferred into 2.0 mL gas chromatography vials, sealed, and stored at −20°C until further analysis.

Fatty acid methyl esters were separated and quantified using a Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) fitted with a flame ionization detector (FID) and a Shimadzu AOC-20i autoinjector. Separation was performed on an Agilent J&W CP-Sil 88 capillary column (100 m × 0.25 mm I.D., 0.20 μm film thickness). Chromatographic conditions included a linear velocity of 20 cm/s, split ratio of 1:10, injector and detector temperatures set at 260°C, and helium carrier gas (Linde Sógas, purity ≥99.999%) at 30 mL/min, with hydrogen and air flow rates of 40 mL/min and 400 mL/min, respectively. The oven temperature was programmed as follows: 100°C held for 5 min, ramped to 180°C at 8°C/min and held for 9 min, then increased to 230°C at 1°C/min and maintained for 1 min.

FAME were identified by comparison with authentic standards and quantified using GC solution software (Shimadzu Corporation, Kyoto, Japan). Fatty acid composition was expressed as a percentage of total TFA, calculated from the ratio of the area of each individual FA peak to the sum of all identified peaks. The absolute content of each FA (mg/g of meat) was determined based on the signal area of the internal standard (tridecanoic acid, C13:0) and its known concentration, according to the following formula:

$$\mathrm{FA}\left(\mathrm{mg}\Big/\mathrm{g\ meat}\right) = \frac{((\mathrm{mg\ C13:0}) \times (\mathrm{FAME\ area}))}{((\mathrm{C13:0\ area})/(\mathrm{g\ meat}))}$$

Lipid quality indices

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

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

The indices of atherogenicity (AI) and thrombogenicity (TI), were estimated as previously proposed (Ulbricht and Southgate, 1991):

$$AI = \left[(C12:0 + (4 \times C14:0) + C16:0)\right] / \left[\left(\sum MUFA + \sum (n-6\ PUFA) + \sum (n-3\ PUFA)\right)\right]$$

$$TI = [(C12:0 + C16:0 + C18:0)] \Big/ \left[\left(0.5 \times \sum MUFA\right) + \left(0.5 \times \sum n-6MUFA\right) + \left(3 \times \sum n-3MUFA\right) + \left(\left(\sum n-3PUFA\right) \Big/ \left(\sum n-6PUFA\right)\right)\right];$$

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated using the equation formerly proposed (Santos-Silva et al., 2002) as follows:

$$h \Big/ H = \frac{(C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3)}{(C14:0 + C16:0)]}$$

The health fatty index (HFI) was calculated using the equation previously proposed (Sanders, 2024) as follows:

$$HFI = \frac{((MUFA \times 2) + (n-6\ PUFA \times 4) + (n-3\ PUFA \times 8) + (n-3/n-6))}{((SFA \times 1) + (MUFA \times 0.5) + (n-6\ PUFA \times 0.25) + (n-3\ PUFA \times 0.125) + (n-6/n-3))}$$

Where SFA MUFA, and PUFA stand for saturated monounsaturated, monounsaturated and polyunsaturated FA, respectively.

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

$$P/S = [(18:2n-6) + (18:3n-3)] / [(14:0 + 16:0 + 18:0)]$$

$$n-6 \Big/ n-3 = \left[\left(\sum n-6\ PUFA\right) \Big/ \left(\sum n-3\ PUFA\right)\right].$$

Statistical analysis

Throughout the results and discussion, the term superiority (expressed as %) was calculated as (maximum value – minimum value)/minimum value, while the term inferiority was calculated as (maximum value – minimum value)/maximum value.

Data on TFA content, FA partial sums, FA ratios, lipid quality indexes, and the individual FA were all submitted to analysis of variance (ANOVA) considering the duck variety as the unique variable, using the GLM procedure of SAS (SAS Inst., Cary, NC, USA; version 9.4). Considering the highly significant differences ($P < 0.001$) observed on TFA content between different duck varieties and their role on FA profile, we executed a second analysis of all data, using the TFA content in muscle as covariate (ANCOVA). The level of statistical significance was set at $P < 0.05$; the least square means and residual standard deviation of the mean (RSD) are presented in tables.

Because TFA content differed significantly among duck types, the FA partial sums of prime FA groups and families were analyzed both as relative content (percentage of TFA) and as absolute content (mg/g fresh meat).

Results and discussion

Fatty acid profile

This study compares meat from Pekin ducks (*Anas platyrhynchos domesticus*) raised under intensive farming conditions with meat from mallard ducks (*Anas platyrhynchos*) obtained from two distinct sources. These samples represent distinct genetic lineages, both between the Pekin and Mallard ducks and within the Mallard populations themselves. In addition, they vary in feeding regimes and life cycles. Given these substantial differences, direct comparisons between the duck meat types should be interpreted with caution, as they limit the scope of data interpretation.

The detailed FA profile, expressed as a percentage of total fatty acid (TFA) content, is shown in Table 1. In total, 24 FAs were identified across the duck meats samples. The wild Mallard duck (WMD) contained

Table 1
Detailed fatty acid (PUFA) profile of breast meat from Pekin Duck raised intensively (PD), Mallard reared in semi-extensive conditions (MDH) and Wild Mallard Duck (WMD) (expressed as % of total FA).

	Duck meat			Statistic	
	PD	MDH	WMD	RSD	P
C12:0	0.975[a]	0.00[b]	0.029[b]	1.243	0.004
C14:0	0.321	0.324	0.300	0.092	0.663
C15:0	0.094[a]	0.065[b]	0.10[a]	0.034	0.007
C16:0	19.56[b]	22.76[a]	17.37[c]	1.248	<0.001
C17:0	0.260[a]	0.096[b]	0.278[a]	0.236	0.003
C18:0	11.70[b]	12.88[a]	11.47[b]	1.095	<0.001
C20:0	0.504[a]	0.180[b]	0.212[b]	0.287	<0.001
C14:1 *cis*-9	0.033[b]	0.027[b]	0.060[a]	0.033	0.006
C16:1 *cis*-9	1.276[b]	2.403[a]	0.945[c]	0.245	<0.001
C17:1 *cis*-9	0.197[b]	0.367[a]	0.000[c]	0.068	<0.001
C18:1 *cis*-9	22.52[c]	35.51[a]	25.61[b]	4.413	<0.001
C18:1 *cis*-11	0.00[b]	2.362[a]	0.036[b]	0.112	<0.001
C20:1 *cis*-11	0.00[c]	0.229[a]	0.040[b]	0.047	<0.001
C18:2 n-6	22.00[a]	12.32[c]	15.98[b]	1.633	<0.001
C20:2 n-6	1.179[a]	0.152[b]	0.288[b]	0.439	<0.001
C20:3 n-6	2.108[a]	0.291[b]	0.395[b]	0.604	<0.001
C20:4 n-6	12.00[b]	7.839[c]	19.25[a]	2.729	<0.001
C22:4 n-6	1.878[b]	1.077[a]	2.014[a]	0.356	<0.001
C22:5 n-6	1.010[a]	0.337[b]	0.456[b]	0.269	<0.001
C18:3 n-3	0.644[b]	0.275[b]	1.879[a]	1.217	<0.001
C20:3 n-3	0.040[b]	0.00[b]	0.326[a]	0.149	0.007
C20:5 n-3	0.163[b]	0.079[b]	0.351[a]	0.295	0.002
C22:5 n-3	0.630[b]	0.154[c]	1.453[a]	0.574	<0.001
C22:6 n-3	0.532[b]	0.265[c]	1.147[a]	0.241	<0.001

Different superscripts in the same row are associated with significant differences ($P < 0.05$);.

23 of these, lacking only margaroleic acid (C17:1 *cis*-9). Whereas both the Pekin duck (PD) and the Mallard duck raised for hunting (MDH) contained 22 FA each. The MDH samples lacked lauric and eicosatrienoic acids (C12:0 and C20:3 n-3, respectively), while PD samples lacked *cis*-vaccenic and gondoic acids (C18:1 *cis*-11 and C20:1 *cis*-11, respectively). Significant differences ($P < 0.01$) were observed among duck meat varieties for the proportion of all identified FAs, with the exception of myristic acid (C14:0), which showed no significant differences ($P = 0.663$).

Table 2 presents the TFA content (expressed as mg/g of fresh meat), the partial sums of major FA groups and families expressed either as absolute values (mg/g of fresh meat) or as relative contents (percentage of TFA), as well as FA ratios (P/S, UFA/SFA, n-6/n-3, h/H, n-6 LCPUFA/n-3 LCPUFA) and lipid quality indices (AI, TI, PI and HFI) for the three duck meat varieties under study. Highly significant differences ($P < 0.001$) were observed in TFA content, all FA group sums (irrespective of whether expressed in absolute or relative terms), as well as in all FA ratios and lipid quality indices. Diet, age and genetics are widely recognized as key factors influencing the FA content and profile of meat (Cobos et al., 2000; Onk et al., 2019; Kokoszyński et al., 2019; Zhang et al., 2022; Wasilewski et al., 2023), and the three duck meat types examined differ across all these factors.

The breast meat of the WMD contained a significantly higher TFA content than the other two duck varieties, being 2.8 times greater than that of the Pekin duck (PD) and 2.5 times greater than that of the MDH. When expressed as absolute content (mg/g of fresh meat), the WMD showed the highest contents of all FA partial sums, differing significantly from both PD and MDH ($P < 0.001$). The PD variety showed the lowest values of total saturated and monounsaturated FA (SFA and MUFA, respectively), differing significantly from the other two varieties in analysis. The MDH presented the lowest total polyunsaturated FA (PUFA) and lowest total long-chain polyunsaturated FA (LCPUFA) (i.e., those PUFA with $\geq$ 20 carbon atoms), embracing both n-6 and n-3 families, differing significantly from both PD and WMD. Conversely, the WMD displayed the highest contents of total n-3 PUFA and total n-3 LCPUFA, differing significantly ($P < 0.05$) from both PD and MDH.

However, when expressed as relative content, MDH differed significantly from both WMD and PD in all FA partial sums. It exhibited the highest percentages of total SFA and total MUFA (36.3% and 40.9% of TFA, respectively), while presenting the lowest levels of PUFA (22.8% of TFA) and total LCPUFA (10.2% of TFA), including both n-6 and n-3 PUFA families. Within the intramuscular FA composition, LCPUFA, are of particular importance, as they include arachidonic (20:4 n-6), eicosapentaenoic (EPA; 20:5 n-3), and docosahexaenoic (DHA; 22:6 n-3) acids, which are involved in many metabolic and physiological processes. These FA play key roles in regulating membrane flexibility, permeability, and receptor function (Calder, 2012; Nakamura et al., 2014; Van Dael, 2021). Analysis of both absolute and relative FA contents, revealed that WMD and MDH displayed the highest and lowest contents of total LCPUFA, as well as total n-6 and n-3 LCPUFAs, respectively. These differences were statistically significant ($P < 0.001$) between them and also in comparison with PD, which exhibited intermediate values but still differed significantly ($P < 0.05$) from extreme values.

An analysis of covariance (ANCOVA) was performed using TFA content as a covariate to determine whether the observed differences in

Table 2
Total FA content (expressed as mg/g of fresh meat), FA partial sums (expressed either as % of total FA and as mg/g of fresh meat), FA ratios and lipid quality indexes of breast meat from Pekin Duck raised intensively (PD), Mallard reared in semi-extensive conditions (MDH) and Wild Mallard Duck (WMD).

	Duck meat			Statistic	
	PD	MDH	WMD	RSD	P
Total FA	7.96[b]	9.19[b]	22.52[a]	2.989	<0.001
Partial sums (expressed as % of total fatty acids)					
Σ SFA	33.41[b]	36.31[a]	29.77[c]	1.362	<0.001
Σ MUFA	24.03[b]	40.90[a]	26.69[b]	4.471	<0.001
Σ PUFA	42.56[a]	22.79[b]	43.54[a]	4.219	<0.001
Σ n-6 PUFA	40.18[a]	22.02[b]	38.38[a]	3.623	<0.001
Σ n-3 PUFA	2.01[b]	0.77[c]	5.16[a]	1.779	<0.001
Σ LCPUFA	19.54[b]	10.19[c]	25.68[a]	3.598	<0.001
Σ n-6 LCPUFA	18.18[b]	9.70[c]	22.40[a]	3.325	<0.001
Σ n-3 LCPUFA	1.37[b]	0.50[c]	3.28[a]	0.824	<0.001
Partial sums (expressed as mg/g of fresh meat)					
Σ SFA	2.68[c]	3.32[b]	6.67[a]	1.057	<0.001
Σ MUFA	1.91[c]	3.86[b]	6.08[a]	1.338	<0.001
Σ PUFA	3.37[b]	2.01[c]	9.77[a]	1.233	<0.001
Σ n-6 PUFA	3.21[b]	1.94[c]	8.53[a]	0.934	<0.001
Σ n-3 PUFA	0.16[b]	0.07[b]	1.16[a]	0.416	<0.001
Σ LCPUFA	1.56[b]	0.94[c]	5.76[a]	0.611	<0.001
Σ n-6 LCPUFA	1.45[b]	0.89[c]	5.02[a]	0.509	<0.001
Σ n-3 LCPUFA	0.11[b]	0.05[b]	0.74[a]	0.178	<0.001
Fatty acid ratios & Lipid Quality Indexes					
P/S[1]	0.719[a]	0.350[c]	0.613[b]	0.078	<0.001
UFA/SFA[2]	2.00[b]	1.76[c]	2.36[a]	0.119	<0.001
n-6/n-3[3]	19.99[b]	28.60[a]	7.44[c]	6.277	<0.001
n-6LCPUFA/n-3LCPUFA[4]	13.27[b]	19.40[a]	6.83[c]	4.348	<0.001
h/H[5]	2.950[b]	2.454[c]	3.758[a]	0.229	<0.001
AI[6]	0.053[a]	0.058[a]	0.031[b]	0.020	<0.001
TI[7]	0.810[b]	1.064[a]	0.615[c]	0.077	<0.001
PI[8]	100.7[b]	55.84[c]	131.2[a]	14.54	<0.001
HFI[9]	3.058[b]	1.914[c]	4.039[a]	0.428	<0.001

Different superscripts in the same row are associated with significant differences ($P < 0.05$); RSD stands as Residual Standard Deviation of the mean; Σ SFA, Σ MUFA and Σ PUFA stand for total saturated, monounsaturated and polyunsaturated fatty acids, respectively; Σ LCPUFA stand for the sum of all PUFA with $\geq$ 20 carbon atoms;.

[1] P/S = [(18:2 n-6 + 18:3 n-3) / (14:0 + 16:0 + 18:0)];.
[2] UFA/SFA = [(MUFA+PUFA) /SFA];.
[3] n6/n3= [(∑n-6) / (∑n-3)];.
[4] n-6LCPUFA/n-3LCPUFA= [(∑n-6LCPUFA) / (∑n-3LCPUFA)];.
[5] h/H (hypocholesterolaemic/hypercholesterolaemic ratio);.
[6] AI (atherogenicity index);.
[7] TI (thrombogenicity index);.
[8] PI (peroxidability index);.
[9] HFI (Healthy fatty index);.

individual FA, FA partial sums and ratios and lipid quality indices were influenced by variations in TFA levels stored in the meat. The results of the ANCOVA, indicate that the differences observed among duck meat varieties are independent of total lipid content and instead reflect intrinsic characteristics of the duck varieties.

Despite differences between duck meat varieties, the predominant FAs were consistent across all meat varieties. Oleic and palmitoleic acids (C18:1 *cis*-9 and C16:1 *cis*-9) were the predominant MUFAs, palmitic and stearic (C16:0 and C18:0) were the principal SFAs, while linoleic and arachidonic acids (C18:2 n-6 and C20:4 n-6) were the main PUFAs.

Together, the six predominant FAs accounted for 89.1–93.7% of total FAs, which is consistent with the results previously published for both Pekin ducks and Mallards (Skrabka et al., 2006; Banaszak et al., 2020; Fan et al., 2020; Zhang et al., 2022; Dal Bosco et al., 2024).

The analysis of data shows that the higher proportion of SFA observed in MDH was primarily driven by increased levels of palmitic and stearic acids. Similarly, the elevated MUFA content in this group was associated with higher contents of C16:1 *cis*-9, C17:1 *cis*-9, C18:1 *cis*-9, C18:1 *cis*-11 and C20:1 *cis*-11 (palmitoleic, margaroleic, oleic, cis-vaccenic and gondoic acids, respectively). These findings suggest enhanced *de novo* synthesis in MDH group compared to the other groups (Fan et al., 2020). This hypothesis is further supported by the management practices applied to these mallards. They are reared outdoors, with free access to multiple ponds for feeding throughout the day, and guided back to rearing sheds in the late afternoon through positive conditioning established during the first month of life, as described in detail (Quaresma et al., 2024). This conditioning is reinforced through the provision of a feed mixture composed of seeds (sorghum, sunflower, corn, barley, oat, and rye in equal proportions), which constitute a natural source of palmitic acid (C16:0). This FA may act as a precursor, directly or indirectly, for many of the SFAs and MUFAs identified. Additionally, several of these feed components, particularly sorghum, corn, barley, oat, and rye, are rich in carbohydrates that can be further converted into FA by *de novo* synthesis, further supporting the observed lipid profile in MDH.

Palmitic acid (C16:0) can be elongated to stearic acid (C18:0) via acyl-CoA C16–C18 elongase (ELOVL6) or through Δ9-desaturation to palmitoleic acid (C16:1 cis-9) by stearoyl-CoA desaturase (SCD). Palmitic acid (C16:0) can be metabolically converted either through elongation to stearic acid (C18:0) via acyl-CoA C16–C18 elongase (ELOVL6) or through Δ9-desaturation to palmitoleic acid (C16:1 cis-9) by stearoyl-CoA desaturase (SCD). Stearic acid may subsequently undergo Δ9-desaturation to form oleic acid (C18:1 cis-9). In turn, palmitoleic and oleic acids can be further elongated to cis-vaccenic acid (C18:1 cis-11) and gondoic acid (C20:1 cis-11), respectively, through the activity of elongase enzymes, primarily ELOVL6 and ELOVL2/ELOVL5. During this elongation process, the position of the double bond shifts from Δ9 (between carbons 9 and 10) to Δ11 (between carbons 11 and 12). The higher content of margaroleic acid (C17:1 cis-9) observed in MDH breast meat may be attributed to de novo synthesis involving the elongation and desaturation of odd-chain FA, such as pentadecanoic acid (C15:0). This pathway leads to the formation of odd-chain monounsaturated FA, including margaroleic acid, which typically occur in low concentrations in animal tissues.

On the other hand, WMD and PD demonstrated a superiority in total PUFA and total n-6 PUFA over MDH, which was achieved by a combination of different individual FA. The WMD presented higher contents of C20:4 n-6, C22:4 n-6, C18:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3, while the PD displayed higher contents of C18:2 n-6, C20:2 n-6, C20:3 n-6 and C22:5 n-6.

Marked differences were observed in the total n-6 and n-3 PUFA profiles between the PD and WMD groups. The PD samples exhibited higher proportions of linoleic acid (C18:2 n-6) and its elongation products (C20:2 n-6, C20:3 n-6 and C22:5 n-6), suggesting a dietary source rich in vegetable oils, which are typically abundant in C18:2 n-6. In contrast, WMD samples showed higher levels of α-linolenic acid (C18:3n-3) and some long-chain PUFAs, from both the n-3 family (C20:3 n-3, C20:5 n-3, C22:5 n-3 and C22:6 n-3) and the n-6 family (C20:4 n-6 and C22:4 n-6).

These findings highlight the strong influence of dietary lipid composition, and contrasting bioavailability between linoleic and α-linolenic acids. The fatty acid profile revealed that PD exhibited greater bioavailability of linoleic acid, whereas WMD showed higher bioavailability of α-linolenic acid. Both linoleic and α-linolenic acids are essential FA and serve as precursors of the long chain FA of the n-6 and n-3 families, respectively. Their metabolic pathways share and compete for the same elongation and desaturation enzymes (Δ6-desaturase, elongase, Δ5-desaturase), leading to competition between the two FA families for the same enzymatic pathways. Therefore, the relative availability of these precursors determines the metabolic flux toward either n-6 or n-3 long-chain PUFA synthesis (Simopoulos, 2008; Smink et al., 2012), with potential divergent effects on human health. It is known that high linoleic acid levels in the diet result in low n-3 LCPUFA status (Gibson et al., 2011).

On the other hand, the WMD exhibits a higher percentage of arachidonic (C20:4, n-6) and adrenic acids (C22:4, n-6), suggesting an increase phospholipid content. This is consistent with the role of arachidonic acid as a principal FA in phospholipids while adrenic acid represents an elongated storage or reserve form of arachidonic acid (Cobos et al., 2000; Hanna and Hafez, 2018). Previous studies have also reported that mallards tend to accumulate greater levels of PUFAs than Pekin ducks, particularly the arachidonic acid (Fan et al., 2020). This tendency may be considered a nutritional advantage for human health, given that arachidonic acid serves as a precursor to a diverse array of bioactive metabolites that are fundamental to health and physiological well-being, particularly though their pivotal roles in immune system regulation (Calder, 2015; Tallima and El Ridi, 2017; Hanna and Hafez, 2018).

Moreover, the elevated levels of elongated and desaturated n-3 and n-6 derivatives in WMD may reflect enhanced Δ5- and Δ6-desaturase and elongase activities, thereby promoting more efficient bioconversion of precursor FA. These differences may be influenced by genetic or physiological factors (e.g., breed, age, or metabolic rate), which could also contribute to these metabolic differences. Overall, the results suggest that both dietary lipid sources and endogenous FA metabolism play key role in shaping the PUFA profile of duck tissues.

Nevertheless, the breast muscle fiber composition may also contribute to the observed variations. The breast meat comprises two muscles, the *pectoralis major* and *pectoralis minor*, which function complementarily during wing flapping. Skeletal muscle is composed of varying proportions of three main fiber types: I (slow-twitch oxidative fibers that use lipid stores as energy reserves); IIa (Fast-twitch oxidative-glycolytic fibers, these fibers can switch between aerobic and anaerobic pathways depending on the demand) and IIb (fast-twitch glycolytic oxidative fibers that use glycogen stores as energy) (Klont et al., 1998; Lefaucheur and Gerrard, 2000). Muscle tissue exhibits considerable plasticity, adapting its structure according to its functional role in locomotion (Torrella et al., 1996). In both WMD and PD, the *pectoralis major* and *minor* muscles are predominantly constituted by type IIa (fast oxidative glycolytic) and IIb (fast glycolytic) fibers (Torrella et al., 1996; Janiszewski et al., 2018; Huo et al., 2021). These fiber types differ in several key aspects. First, their cross-sectional area, influences the relative contribution of the sarcolemma to total fiber mass, with smaller fibers exhibiting a higher membrane proportion. Second, their metabolic profile determines substrate utilization: type I use chiefly triacylglycerols as energy. type IIa fibers use both triacylglycerols and glycogen as energy, whereas type IIb fibers rely primarily on glycogen. Third, their subcellular organization varies, as type I fibers exhibiting a higher mitochondrial density, leading to a greater proportion of subcellular membranes, and by consequence higher percentage of phospholipids (Alasnier et al., 1996; Klont et al., 1998; Chizzolini et al., 1999; Dinh et al., 2011).

Differences in muscle fiber types and different proportions of the three muscle fibers in different muscles or meat portions may account for differences observed between breast and leg meat portions in a single animal, as previously suggested (Quaresma et al., 2016a, 2022, 2024, 2025; Antunes et al., 2019).

Nutritional assessment of duck meat varieties

In duck meat, phospholipids account for approximately 50% of the total lipids (Wang et al., 2009), however, this proportion may vary between Mallards and Pekin ducks, and even among the Mallard populations in the study, considering differences in: 1) the lipid reserves in the breast muscle and; 2) the muscle fiber composition. Regardless, of their classification as polar or neutral lipids, FA play essential roles in human metabolism and are closely linked to various health outcomes (Chen and Liu, 2020), Consequently, several FA ratios and lipid quality indices have been developed to assess the nutritional value of foods and diets. Recommended values have been established for the polyunsaturated-to-saturated fatty acid ratio (P/S $>$ 0.45), unsaturated-to-saturated fatty acid ratio (UFA/SFA $>$ 2.0), n-6/n-3 ratio ($<$ 4.0), atherogenicity index (AI $<$ 1.0), thrombogenicity index (TI $<$ 1.0), and hypocholesterolemic-to-hypercholesterolemic fatty acid ratio (h/H $>$ 2.0) Ulbricht and Southgate, 1991; British Department of Health, 1994; Simopoulos, 2002, 2008; Santos-Silva et al., 2002; WHO, 2023). According to these recommendations, the P/S, UFA/SFA, and h/H ratios, as well as the AI and TI indices, of duck meat from both the PD and WMD groups were within the recommended ranges, indicating a favorable lipid nutritional profile. On the other hand, the P/S, UFA/SFA ratios and the AT index of meat from MDH were out of the recommended values. Whereas, the n-6/n-3 ratio was quite above the recommend value in all three duck varieties in comparison. However, the n-6/n-3 recommendations are outdated, once the 2010 FAO/WHO Expert Consultation (FAO, 2010) shifted away from recommending a specific n-6:n-3 ratio, concluding that available evidence was insufficient to establish a single optimal value. Consequently, they emphasized that priority should be given to achieving adequate absolute intakes of individual n-3 and n-6 fatty acids.

Nevertheless, WMD exhibited the most favorable lipid profile, characterized by the highest h/H and HFI ratios, the lowest atherogenicity and thrombogenicity indices, and the lowest n-6/n-3 and n-6LCPUFA/n-3LCPUFA ratios, all indicative of superior nutritional quality. On the other hand, MDH showed the least favorable profile, characterized by the highest P/S, h/H and HFI values, alongside the highest atherogenicity and thrombogenicity indices (AI and TI) and the highest n-6LCPUFA/n-3LCPUFA and n-6/n-3 ratios. Collectively, these characteristics result in the poorest nutritional quality. From a nutritional perspective, PD ranked intermediately among the evaluated duck types, presenting generally balanced indices; however, although its relatively high P/S ratio is advantageous, the simultaneously elevated n-6/n-3 values may represent a limitation.

The analysis of LCPUFA) revealed marked differences among duck types, with WMD and MDH showing the highest and lowest total LCPUFA, respectively (25.7% vs. 10.2% of TFA), while PD displayed intermediate levels (19.9% of TFA). Particular attention is given to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two n-3 LCPUFA that are indispensable structural and functional components of cell membranes. These FA play key roles in modulating inflammatory responses, lipid metabolism, and neuronal signalling. Due to their well-documented benefits for cardiovascular, cognitive, and metabolic health, EPA and DHA are recognized as critical dietary nutrients. Their importance is further underscored by the limited capacity of humans to synthesize them endogenously, making dietary intake essential.

Therefore, Global health organizations have established nutritional recommendations on the daily intake of EPA plus DHA. The International Society for the Study of Fatty Acids and Lipids (ISSFAL), the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the Academy of Nutrition and Dietetics of USA (AND) recommend a daily intake of 500 mg/day of EPA + DHA for the general adult population to reduce cardiovascular disease risk in the general population. On the other hand, the European Food Safety Authority (EFSA) recommends a lower intake of 250 mg/day of combined EPA and DHA for adults to maintain normal cardiac function and overall cardiovascular health, with an additional recommendation of 250 mg/day of DHA for optimal brain function and vision.

Across the duck meat varieties examined, total n-3 LCPUFA content ranged from 4.6 to 73.9 mg/100 g, with EPA+DHA levels varying between 3.2 to 33.7 mg/100 g of fresh meat. Based on these values, a 100 g serving of WMD, MDH and PD meat would provide approximately 13.5%, 1.3%, and 2.2%, respectively, of the EFSA-recommended intake of EPA + DHA.

Discriminatory ability of the intramuscular fatty acid profile

The canonical discriminant analysis (CDA) combined with FA profile, is widely used to differentiate veal and beef from different production systems (Dias et al., 2008; Alfaia et al., 2009) and meats from different certification labels (Monteiro et al., 2012; Quaresma et al., 2016b). In this study, CDA of intramuscular FA composition was applied to discriminate among three duck meat varieties.

A forward stepwise procedure identified the most relevant predictors, selecting 12 out of the 24 FA as having the greatest discriminatory power. This approach generated two canonical functions that maximized between-group variance while minimizing within-group variability. The first canonical function (Root 1) explained 79.3% of the total variance, and was primarily associated with *cis*-vaccenic acid (C18:1 *cis*-11) which showed the highest discriminant loading (0.8858). The second function (Root 2) accounted for the remaining 20.7% of the variance and was mainly driven by linoleic acid (C18:2 n-6), with the highest loading (0.9109). The correlation matrix loadings and discriminant functions are presented in Table 3. Together, these two canonical functions provided a clear separation among the three duck varieties (Figure 1).

The model achieved 100% correct classification of all 84 samples—47 PD (58.02%), 14 MDH (17.28%), and 20 WMD (24.69%)—indicating excellent discriminatory performance (Table 4). This result was fully confirmed by leave-one-out cross-validation, which also yielded 100% classification accuracy. For variety differentiation, the two canonical discriminant functions were selected (Figure 1). The recognition ability of the discriminant model was evaluated by the correct classifications of 100% during the modelling step, allowing the differentiation of the three duck varieties. The analysis of Figure 1 shows that PD samples clustered tightly in the upper-left quadrant, MDH in the

Table 3
- Results of the canonical discriminant analysis: loadings of correlation matrix between predictor variables (standardized canonical coefficients) and discriminant functions (roots 1, 2, 3 and 4), and some statistics for each function.

Variables	Root 1	Root 2
C14:0	−0.2499	−0.9228
C16:0	0.4137	0.6090
C18:0	0.4961	0.6685
C16:1 *cis*-9	0.5650	0.1296
C17:1 *cis*-9	0.5120	0.8772
C18:1 *cis*-11	0.8858	−0.1083
C20:1 *cis*-11	0.1836	−0.3686
C18:2 n-6	−0.1828	0.9109
C20:3 n-6	−0.1086	0.5712
C20:4 n-6	−0.1491	−0.6909
C20:3 n-3	0.1480	0.5907
C22:6 n-3	−0.1798	−0.4520
Statistics		
Canonical R	0.9955	0.9831
Eigenvalue	110.7131	28.8963
Cumulative proportion	0.7930	1.0000
Probability	<0.0001	<0.0001

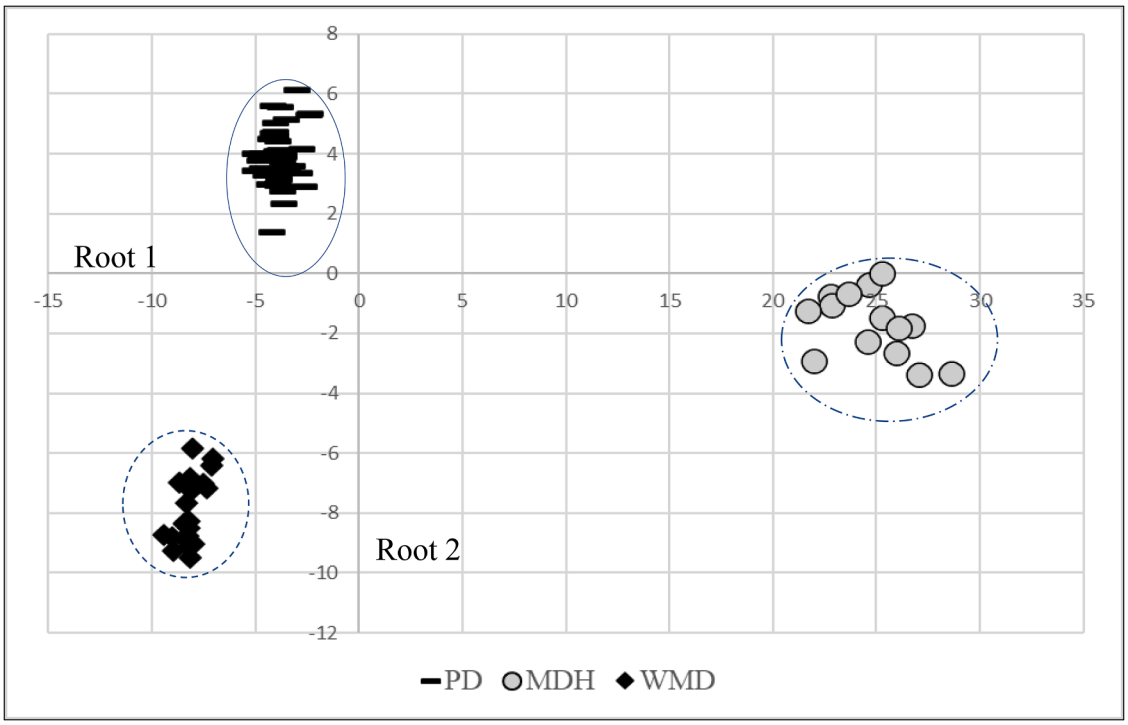

Fig. 1. Plot of the discriminant functions (root 1 versus root 2) for the classification of duck meat varieties.

Table 4
Classification matrix of cross-validation for duck meat varieties (number of observations and percent classified into variety).

Duck meat variety	Number of observations and (percentage classified into variety)			
	PD	MDH	WMD	Total
PD	47 (100%)	0	0	47 (100%)
MDH	0	14 (100%)	0	14 (100%)
WMD	0	0	20 (100%)	20 (100%)
Total	47 (58.02%)	14 (17.28%)	20 (24.69%)	81 (100%)

PD stands for Pekin Duck raised intensively; MDH stands for Mallard reared in semi-extensive conditions and WMD stands for Wild Mallard Duck.

upper-right quadrant, and WMD in the lower-left quadrant. The clear separation of the clusters with no overlap highlights the strong discriminatory power of the FA profiles, while the tight within-group clustering indicates high similarity among samples of the same variety.

The CDA model achieved 100% correct classification for all 84 samples: 47 PD (58.02%), 14 MDH (17.28%), and 20 WMD (24.69%) (Table 4). Leave-one-out cross-validation confirmed the model's robustness, with all samples correctly assigned to their respective variety, demonstrating the FA profile's strong discriminatory ability.

Conclusions

The evaluated duck varieties exhibited distinct intramuscular fatty acid (FA) profiles. WMD showed the most favorable lipid profile (higher PUFA, n-3 PUFA, and n-3 LCPUFA) and better lipid quality indices. Conversely, MDH had higher SFA and MUFA contents, reflecting increased *de novo* lipogenesis and lower nutritional quality, while PD displayed intermediate traits. FA variability seemed to be influenced by dietary factors, endogenous metabolism (precursor availability and elongation/desaturation activity), muscle fiber composition, and lipid partitioning.

Although beneficial FAs like EPA and DHA were present, their low concentrations in these duck meats disqualify them to be considered a primary dietary source of these nutrients.

Canonical discriminant analysis achieved 100% classification accuracy, demonstrating that intramuscular FA profiles are highly effective for product authentication and variety differentiation of duck meat.

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Generative AI statement

During the writing of this article, the authors used ChatGPT (OpenAI) for writing improvement. After using the ChatGPT, the authors reviewed and edited the content as necessary and take full responsibility for the content of the publication.

CRediT authorship contribution statement

M.A.G. Quaresma: Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization. **M.L. Nunes:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **G. Pereira:** Software, Data curation. **R. Batarda:** Visualization, Investigation. **F. Abade dos Santos:** Visualization, Resources, Investigation. **D. Rodrigues:** Resources, Methodology, Investigation. **C Roseiro:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation. **M.L. Galante:** Visualization, Validation, Methodology, Investigation. **S.L. Morais:** Visualization, Validation, Methodology, Investigation. **C. Delerue-Matos:** Writing – review & editing, Validation, Supervision, Funding acquisition, Data curation. **V. F. Domingues:** Writing – review & editing, Validation, Supervision,

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