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The influence of diet supplementation with oat hay and whole carrot on the composition of rabbit meat lipid fraction

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ABSTRACT

Reducing feeding costs without compromising rabbit health, productivity, and meat quality is essential to increase the competitiveness of rabbit meat. This study tested the hypothesis that supplementing rabbits' diet with oat hay and whole carrots, either singly or in combination, does not affect the composition and nutritional quality of the meat's lipid fraction.

Eighty rabbits were divided into four groups: control (CC, fed concentrate feed exclusively), CT (whole carrot supplementation), OH (oat hay supplementation), and CO (whole carrot and oat hay). In the CC group, concentrate feed supplied 66.0 g/rabbit of total fat, decreasing by 22.8 % and 22.6 % with carrot or oat hay supplementation alone, and by 39.2 % when both were combined. Conversely, supplementation added 6.14, 18.96, and 25.1 g/rabbit of total fat in the CT, OH, and CO groups, respectively.

The *longissimus thoracis et lumborum* (LTL) muscles used in this trial were sampled following complete processing of the rabbits, and their fatty acid profile remained mostly unchanged. Oat hay supplementation increased the proportion of odd-numbered and branched-chain fatty acids plus the C18 biohydrogenation intermediates ($P = 0.024$) and reduced the contents of total cholesterol by 26.5 % ($P = 0.003$) and total vitamin E by 56.3 % ($P < 0.001$). Additionally, CO supplementation improved the n-3 PUFA percentage and decreased the n-6/n-3 ratio in the LTL muscles. In conclusion, oat hay and carrot supplementation induced minor lipid profile changes but showed potential nutritional benefits, such as lower cholesterol and improved fatty acid balance. However, the supplementation strategies tested reduced total vitamin E contents in rabbit meat, potentially reducing oxidative stability.

1. Introduction

Due to good productive traits such as high prolificity, short gestation length, rapid growth, and efficient feed utilization, the domestic rabbit (*Oryctolagus cuniculus*) is considered a promising species for meat production (Dalle Zotte, 2014). Moreover, rabbits can be primarily fed on forages and agricultural by-products (Cheeke, 1986), highlighting their strong potential for production without competing with human food resources. The domestic rabbit is a non-ruminant herbivorous, and hindgut fermenter species, exhibiting cecotrophic behavior (Carabaño et al., 2010; Irlbeck, 2001). The microbiota housed in the caecum is able to hydrolyze dietary fiber, and synthesize important nutrients for the rabbit's nutrition, these nutrients are packed in cecotrophs (also known as soft feces), which are ingested directly from the anus, allowing the rabbit to recycle these nutrients (Gidenne et al., 2010). The cecotrophs are rich in microbial protein with high contents of essential amino acids, and possess B-complex and K vitamins (Lebas, 2000).

Rabbit meat is nutritionally advantageous, offering low fat and cholesterol levels, a balanced fatty acid profile, despite its high content in n-6 polyunsaturated fatty acids (Dal Bosco et al., 2014; Siudak & Kowalska, 2024). It also provides a wide range of essential nutrients such as B-complex vitamins, minerals and trace elements (Combes,

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2004; Dalle Zotte, 2002, 2014; Dalle Zotte & Szendro, 2011; Rasinska et al., 2018). Therefore, rabbit meat is widely recognized for its high nutritional value and health benefits, and is also regarded as hypoallergenic (Petraci & Cavani, 2013; Takahata et al., 2000). Although rabbits possess desirable production traits and meat quality, their meat remains more expensive than broiler meat due to higher labor cost and the reliance on concentrate-based feeding systems (Dalle Zotte, 2002).

Therefore, lowering feed expenses and embracing sustainable practices, particularly through the use of forages and agro-industrial residues in the formulation of concentrate feeds for rabbits, is essential for improving the economic viability of rabbit farming. Following this approach, we conducted a trial to evaluate the effects of supplementing concentrate-fed rabbits with oat hay, whole carrots, or both on productive performance (Quaresma et al., 2023). Besides productive performance and production costs, meat quality is a critical determinant of the success of any dietary strategy. This study aimed to investigate whether supplementing rabbit diets with oat hay and/or carrots influences the composition and nutritional quality of meat lipid fraction.

2. Materials and methods

2.1. Ethics statement

The trial was conducted in compliance with national legislation (Decree-Law No. 113/2013), which incorporates Directive 2010/63/EU of the European Parliament and Council. The protocol was approved by the Animal Welfare Body (ORBEA) of the Faculty of Veterinary Medicine, University of Lisbon (Ref. 003/2023).

2.2. Experimental design, diet composition and feeding management

The experimental trial was carried out in a commercial rabbitry (Agrodunas Lda, Cantanhede), under commercial production conditions, i.e., those regularly used in the grow-out-fattening unit, namely: temperature between 16 and 22 °C, average relative humidity of 60 %; and a 12 h photoperiod, adjusted to the light cycle (7.30 h - 19.30 h). Eighty Hyla 2000 hybrid rabbits (Eurolap, Gosne, France) were selected at weaning (30 days old) from ten independent litters, all born on the same day, each with at least ten healthy kits. From each litter, eight kits were chosen (maintaining a 1:1 sex ratio), selecting those closest to the average weaning weight and excluding the lightest and heaviest individuals.

The selected littermates were randomly distributed by the four experimental groups (2 littermates/experimental group with a sex ratio of 1/1), and tattooed for identification purposes in their left ear. Therefore, each experimental group encloses 2 littermates from 10 independent litters, a total of 20 rabbits was allocated to 5 topped wire cages with wire-mesh floor (4 rabbits per cage with 0.5 m^2). The cages included automatic feeders and drinkers. The concentrated feed was provided ad libitum at the feeders, while supplementation with oat hay or/and whole carrot (referred hereafter as carrot) was provided on the cage's floor in the corner adjacent to the feeder.

Regarding dietary management, rabbits in all four experimental groups were fed with concentrate feed. The control group (CC group) was fed concentrate feed exclusively; the CT group was supplemented with whole carrots (50 g of carrots/rabbit/day); the OH group was supplemented with oat hay (30 g of oat hay/rabbit/day); the CO group was supplemented with oat hay and whole carrots (30 g of oat hay plus 50 g of whole carrots/rabbit/day). Detailed information on the proximate composition of the concentrate, carrot, and oat hay, along with the ingredient and nutrient profile of the concentrate feed, is available in Quaresma et al. (2023). The trial began immediately after weaning at 30 days of age and ended at slaughter on day 68. At 36 days of age, rabbits were weighed, showing an average body weight of 1113 ± 95.4 g (mean ± SD), with no significant differences among groups ($P = 0.90$).

Throughout the experimental period, rabbits in the CT, OH, and CO groups consumed a total of 1600 g/rabbit of whole carrot, 960 g/rabbit of oat hay, and 1600 g of whole carrot plus 960 g of oat hay per rabbit, respectively. On a dry matter (DM) basis, this corresponded to 193.6 g of whole carrot DM per rabbit, 790.1 g of oat hay DM.

per rabbit, and 983.7 g of combined DM per rabbit. Supplementation with whole carrot or oat hay presented individually reduced concentrate intake by 579 g and 573 g of DM per rabbit, respectively, whereas their combination resulted in a reduction of 996 g of DM. Consequently, carrot supplementation alone or in combination with oat hay decreased total DM intake by 385.1 g/rabbit and 12.6 g/rabbit, respectively, whereas oat hay supplementation alone increased total DM intake by 216.8 g/rabbit.

In the CC group, concentrate feed provided 394.9 g/rabbit of total protein, decreasing by 15.9 % and 6.3 % with carrot or oat hay supplementation alone, and by 16.1 % when carrot and oat hay were combined. Regarding total fiber supplied, concentrate feed delivered 428.8 g/rabbit of total fiber, supplementation with carrot and hay, when provided individually, increased the total dietary fiber content by 19.4 %% and 47.9 %%, respectively; however, this increase reached 73.4 %% when both were administered in combination.

In the CC group, concentrate feed supplied 66.0 g/rabbit of total fat, decreasing by 22.8 % and 22.6 % with carrot or oat hay supplementation alone, and by 39.2 % when both were combined. Conversely, supplementation added 6.14, 18.96, and 25.1 g/rabbit of total fat in the CT, OH, and CO groups, respectively.

2.3. Slaughter and meat sampling

All rabbits used in the trial were slaughtered on a single day by electrical stunning at the Litoral Coelho abattoir (Tocha, Portugal), in full compliance with EU legislation. Meat sampling was performed after complete carcass processing and involved the removal of the entire thoracolumbar section from each rabbit, which were individually bagged, labeled, and transported under refrigeration to the laboratory (approx. 3 h). In the laboratory, the left and right *longissimus thoracis* et *lumborum* muscles (hereafter mentioned as LTL muscle) were excised, rimmed of visible connective tissue, ground individually using a domestic food processor (Moulinex, France), vacuum-sealed in polypropylene bags, and stored at −20 °C until analysis, which was completed within one month.

2.4. Chemicals and reagents

HPLC grade n-hexane was purchased from Merck (Darmstadt, Germany) and absolute ethanol (99.8 % *v*/v) from AGA (Lisboa, Portugal). High-purity gases, R grade (helium, hydrogen, nitrogen and air) were acquired from Gasin (Gasin Grupo Air products, Lisboa, Portugal). A standard mixture of 37 fatty acid methyl esters (FAMEs) was obtained from Supelco (Bellefonte, PA, USA) and used for peak identification. Standard solutions of tocopherols (α, β, γ, and δ) for vitamin E analysis were sourced from Calbiochem (La Jolla, CA, USA) and diluted in n-hexane. Cholesterol standard (> 99.0 % purity) was supplied by Sigma-Aldrich (Steinheim, Germany). Ultrapure water was produced using a Milli-Q system (Millipore, Bedford, MA, USA). Boron-trifluoride (14 %) in methanol, and all other chemicals used were of analytical grade.

2.5. Fatty acid methyl esters analysis

To obtain the fatty acid (FA) profile, direct transesterification of edible meat into FAME was performed according to the methodology developed by Rule (1997). Briefly, lyophilized meat samples (0.100 g) and 100 μl of internal standard solution (nonadecanoate acid in n-hexane; 2 mg/ml) were mixed with 2 mL of 14 % boron-trifluoride in methanol (Sigma-Aldrich, Steinheim, Germany), and then the mixture was vortexed for 1 min and incubated in a water bath at 80 °C for 2 h under constant stirring. The resulting FAMEs were analyzed by gas

chromatography (Shimadzu GC-2010 Plus; Kyoto, Japan) equipped with a flame ionization detector (FID). One µl of sample was injected and separation was achieved using a SP-2560 capillary column (100 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco, Bellefonte, PA, USA), 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 20 min, increased again at 1 °C/min to 190 °C and finally increased at 2 °C/min to 220 °C, maintained for 40 min. The injector and detector temperatures were maintained at 250 °C and 280 °C, respectively. Fatty acids were identified by comparing retention times with those of known commercial standards (Supelco, Bellefonte, PA, USA) and reference chromatograms (Alves & Bessa, 2009).

The intramuscular FA profile was expressed either as absolute content (mg/100 g of meat) or as relative content (% of total FA content). The absolute values (mg/100 g meat) reflect the fatty acid concentration in the edible portion, calculated based on the known concentration and peak area of the internal standard (C19:0), using the following equation:

$$FA\ (mg/100\ g\ meat) = 100\ x\ \left(\frac{(C19:0\ g) \times (FAME\ area)}{(C19:0\ area) \times (meat\ g)}\right)$$

FAMEs from both oat hay and carrot were obtained using a modified version of the method by Sukhija and Palmquist (1988). The chromatographic conditions (GC-FID) applied were consistent with those used for the analysis of meat samples. The fatty acid profiles of oat hay and carrot are reported in Table 1, expressed both as percentage of total FA and as mg/g of dry matter.

2.6. Total cholesterol and vitamin E analysis

Total cholesterol (TC) and vitamin E (sum of all natural tocochromanols) were quantified in duplicate, according to the protocol described by Prates et al. (2006).

In summary, 0.75 g of the LTL muscle was weighed into a screw-capped Teflon-lined tube, followed by the addition of 0.2 g of L-ascorbic acid and 5.5 ml of freshly prepared saponification solution (11 % *w/v* KOH in ethanol:deionized water, 55/45 *v/v*). To prevent oxidation, the tube was flushed with nitrogen prior to mixing. After complete dissolution of the ascorbic acid, the mixture was subjected to saponification in a shaking water bath at 80 °C for 15 min (200 rpm). Samples were then cooled, and the unsaponifiable fraction extracted with n-hexane (containing 25 µg BHT/ml), followed by centrifugation (1500 *g*, 5 min) to facilitate phase separation. An aliquot of the organic (n-hexane) phase was transferred to a screw-capped Teflon-lined tube containing a small amount of anhydrous sodium sulfate, gently agitated, and filtered through a 0.45 µm hydrophobic membrane into amber glass vials with Teflon-sealed caps.

Table 1
The fatty acid profile of both oat hay and whole carrot (expressed as % of total fatty acid content and as mg/g of dry matter).

	Oat hay		Whole carrot	
	% of total FA	mg/g of DM	% of total FA	mg/g of DM
Total FA	100	6.110	100	20.124
C14:0	1.454	0.089	- -	- -
C15:0	0.697	0.043	- -	- -
C16:0	33.586	2.052	18.734	3.770
C18:0	4.765	0.291	1.817	0.366
C20:0	2.147	0.131	0.809	0.163
C22:0	3.927	0.240	1.245	0.251
C18:1 *cis*-9	11.176	0.683	4.918	0.990
C18:1 *cis*-11	0.890	0.054	0.525	0.106
C18:2 n-6	25.399	1.552	66.788	13.440
C18:3 n-3	15.960	0.975	5.165	1.039
Total SFA	46.576	2.846	22.605	4.550
Total MUFA	12.066	0.737	5.443	1.096
Total PUFA	41.359	2.527	71.953	14.479

DM – Dry matter; SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids.

Post-saponification, the purified extracts were analyzed using an HPLC system (Alliance 2695, Waters, Milford, USA) equipped with a normal-phase silica column (Nova-Pack Silica 4 µm, 3.9x150mm, Waters, Milford, USA). Tocopherols were detected by fluorescence (Waters 474, Waters, Milford, USA) (excitation at 295 nm, emission at 325 nm), while total cholesterol was quantified using UV detection at 202 nm (Waters 2487 UV/VIS detector, Waters, Milford, USA).

The concentrations of cholesterol and tocopherols in rabbit meat were quantified in duplicate using external standard calibration curves, and only results with a coefficient of variation (CV) below 5 % were accepted. Results are expressed as mg/100 g of meat for total cholesterol, and as µg/g of meat for total vitamin E, α-tocopherol, and γ-tocopherol.

2.7. Lipid quality indices

Lipid indices were calculated according to the literature: the peroxidability index (PI) as described by Arakawa and Sagai (1986), the atherogenic (AI) and thrombogenic (TI) indices according to Ulbricht and Southgate (1991),the hypocholesterolemic/hypercholesterolemic ratio (h/H) as proposed by Santos-Silva et al. (2002), and the P/S and n-6/n-3 ratios according to the British Department of Health (1994). The corresponding equations are as follows:

PI = (% monoenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexaenoic × 8)

AI = [(C12:0 + (4 × C14:0) + C16:0)] / [∑MUFA + ∑(n − 6 PUFA) + ∑(n − 3 PUFA)]

TI = [(C14:0 + C16:0 + C18:0)] / [(0.5 × ∑MUFA) + (0.5 × ∑n − 6 PUFA) + (3 × ∑n − 3 PUFA) + ((∑n − 3 PUFA)/(∑n − 6 PUFA))]

h/H = [(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)

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

n-6/n-3 = ∑n-6 PUFA / ∑n-3 PUFAwhere MUFA and PUFA stands for monounsaturated and polyunsaturated fatty acids, respectively.

2.8. Statistical analysis

The study involved 4 treatments (diets), each randomly assigned to 20 rabbits allocated in 5 cages. Cages were considered the experimental units, while meat samples collected from individual rabbits within each cage were treated as subsamples. Statistical analysis was conducted using the MIXED procedure in SAS (version 9.4; SAS Inst., Cary, NC) with a model that considered the diet as a fixed effect and the cage as a random term.

Whenever a significant difference was detected, a post hoc analysis using the Tukey procedure was conducted to identify significant differences ($P < 0.05$) between the least square means. Statistically significant differences were considered when $P < 0.05$, and the statistical tendency was defined when the P value was between 0.05 and 0.10. The least square means and standard error of the mean (SEM) are presented in tables.

3. Results and discussion

The present study explores the effects of including oat hay and carrot, individually or combined, in rabbit diets as a potential strategy to reduce feeding costs, as previously suggested by Quaresma et al. (2023). The findings indicate that the incorporation of these ingredients can decrease concentrate feed intake and mitigate rabbit meat production costs without significantly compromising productive performance. This study investigates the impact of these dietary interventions on meat composition, specifically evaluating total FA content and profile, total cholesterol levels, and the concentration and profile of vitamin E.

Considering the primary objective of this study was to assess the impact of dietary supplementation on the lipid composition of rabbit

meat, it was essential to accurately determine the animals' total fat intake. In the CC group, concentrate feed contributed with 66.0 g/rabbit of total fat. This contribution was reduced by 22.8 % and 22.6 % following carrot or oat hay supplementation alone, and by 39.2 % when both supplements were provided together. Conversely, supplementation contributed with an additional 6.14 g/rabbit, 18.96 g/rabbit, and 25.1 g/rabbit of total fat in the CT, OH, and CO groups, respectively. The reduction in concentrate feed intake led to a decreased ingestion of lard and its principal constituents, while supplementation contributed with vegetable oils.

3.1. The rabbit meat total fatty acid content and fatty acid profile

The intramuscular fat of rabbit meat includes both polar and neutral lipid components, chiefly phospholipids and triacylglycerols, respectively. In this work, the analysis of the fatty acid profile integrates these two fractions, enabling a more accurate evaluation of the meat's nutritional attributes. Table 2 summarizes the total FA content (mg/100 g of meat), the combined values of key fatty acids (in mg/100 g and as a percentage of total FAs), as well as FA ratios and indices relevant to lipid quality. Tables 3 and 4 provide a detailed composition of individual fatty acids, reported in absolute and relative terms, expressed either as mg/100 g of meat or as % of total FA, respectively.

The total fatty acid (FA) content did not show statistically significant variation among the dietary treatments ($P = 0.111$), averaging 3.73 g/100 g of meat. This result occurred despite differences in concentrate intake between experimental groups, variations in the composition of diet supplements, and the resulting differences in net energy intake, suggesting that the structural fatty acids of phospholipids were the primary determinants of the total fatty acid content observed in rabbit meat. The loin, corresponding to the *longissimus lumborum* muscle, is

Table 2
Total fatty acid content (expressed as mg/100 g of fresh meat) fatty acid partial sums (expressed either as mg/100 g of fresh meat and as % of total FA), nutritional FA ratios, and lipid quality indices of rabbit meat with different feeding management.

	Group				Statistics	
	CC	CT	OH	CO	SEM	*P*
Total FA	4082	3562	3284	3842	225.3	0.111
Partial sums (expressed as mg/100 g of fresh meat)						
Σ SFA	1451	1265	1176	1374	82.8	0.138
Σ SFA lin.	1432	1247	1159	1356	82.0	0.139
Σ SFA br.	18.9	17.6	16.2	18.6	0.83	0.137
Σ MUFA	1192	1032	942	1123	72.2	0.122
Σ MUFA *cis*	1173	1014	925	1105	71.6	0.121
Σ MUFA *trans*	15.9	15.2	14.5	15.9	0.70	0.429
Σ PUFA	1426	1253	1154	1333	72.1	0.091
Σ n-6 PUFA	1348	1182	1089	1256	67.9	0.088
Σ n-3 PUFA	75.8	68.0	62.3	74.0	4.24	0.140
Σ OBCFA	60.7	55.8	51.7	59.6	2.97	0.177
Σ OBCTFA	90.1	84.2	79.2	88.2	3.78	0.234
Σ Others	12.5	12.2	12.4	12.1	0.43	0.870
Partial sums (expressed as % of total FA)						
Σ SFA	35.0	34.9	35.2	35.1	0.26	0.883
Σ SFA lin.	34.5	34.4	34.7	34.6	0.26	0.772
Σ SFA br.	0.46	0.49	0.48	0.47	0.01	0.132
Σ MUFA	28.9	28.6	28.5	28.6	0.27	0.742
Σ MUFA *cis*	28.5	28.2	28.1	28.2	0.28	0.687
Σ MUFA *trans*	0.38	0.41	0.42	0.41	0.01	0.131
Σ PUFA	35.1	35.4	35.1	35.3	0.42	0.968
Σ n-6 PUFA	32.8	33.0	32.7	32.9	0.40	0.968
Σ n-3 PUFA	1.84^{b}	$1.89^{a,b}$	$1.87^{a,b}$	1.92^{a}	0.02	0.022
Σ OBCFA	1.48	1.55	1.57	1.56	0.02	0.062
Σ OBCTFA	2.17^{b}	$2.30^{a,b}$	2.36^{a}	$2.30^{a,b}$	0.04	0.024
Σ Others	0.69	0.75	0.80	0.73	0.04	0.108
Ratios and indices						
P/S	0.99	1.00	0.98	0.99	0.02	0.935
n-6/n-3	17.8^{a}	$17.5^{a,b}$	$17.6^{a,b}$	17.1^{b}	0.18	0.048
h/H	2.20	2.21	2.21	2.21	0.04	0.988
AI	0.56	0.55	0.55	0.56	0.001	0.811
TI	0.82	0.82	0.83	0.82	0.01	0.864
PI	1827	1627	1525	1714	80.8	0.095

Regarding experimental groups, CC stands for Control group; CT stands for carrot supplementation, OH stands for oat hay supplementation and CO stand for carrot plus oat hay supplementation; Different superscripts in the same row are associated with significant differences ($P < 0.05$); SEM – Standard error of the mean; SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids; h/H – hypocholesterolaemic/hypercholesterolaemic ratio; AI – atherogenicity index; TI – thrombogenicity index; PI – peroxidability index.

Table 3
Detailed fatty acid profile in rabbit's meat (expressed as mg/100 g of fresh meat).

	Group				Statistic	
	CC	CT	OH	CO	SEM	P
C10:0	9.75	9.07	9.54	11.1	1.107	0.585
C12:0	27.5	23.3	21.9	27.2	1.723	0.090
C13:0	0.89	0.85	0.85	1.08	0.069	0.070
C14:0	109	92.5	82.8	104	6.961	0.079
C15:0	20.6	19.1	17.9	20.4	1.082	0.288
C16:0	1007	870	806	942	58.96	0.133
C17:0	22.9	20.8	19.1	22.0	1.170	0.166
C18:0	229	206	196	223	12.25	0.245
C20:0	4.45	3.72	3.76	4.25	0.335	0.359
C22:0	1.52	1.55	1.43	1.56	0.131	0.886
i-C14:0	1.56	1.52	1.41	1.67	0.089	0.261
i-C15:0	2.29^{a}	$2.07^{a,b}$	$1.85^{a,b}$	1.24^{b}	0.105	0.039
ai-C15:0	3.46	3.17	3.06	3.46	0.185	0.332
i-C16:0	7.52	6.82	6.24	7.12	0.328	0.086
i-C17:0	1.51	1.43	1.32	1.58	0.082	0.139
Ciclo-17	2.62	2.59	2.27	2.71	0.215	0.506
C14:1 *cis*-9	3.27	2.77	2.42	2.96	0.412	0.534
C16:1 *cis*-7	13.4	11.7	10.9	12.6	0.691	0.110
C16:1 *cis*-9	61.3	51.6	44.6	56.5	6.243	0.316
C17:1 *cis*-9	6.69	6.01	5.32	6.58	0.464	0.188
C18:1 *cis*-9	1080	959	873	876	86.25	0.319
C18:1 *cis*-11	43.9	37.4	36.9	40.2	2.999	0.360
C18:1 *cis*-12	3.34	3.70	3.18	3.62	0.521	0.883
C18:1 *cis*-13	1.13	1.05	1.17	1.01	0.126	0.785
C20:1	9.53	7.71	7.99	8.82	0.867	0.458
C18:1 *trans*-6-8*	1.20	1.03	0.98	1.24	0.134	0.471
C18:1 *trans*-9	5.52	5.03	5.10	4.89	0.277	0.428
C18:1 *trans*-10	0.74	0.51	0.60	0.82	0.102	0.183
C18:1 *trans*-11	4.51	4.17	3.61	4.73	0.459	0.349
C18:1 *trans*-12	4.34	4.05	3.48	3.67	0.238	0.066
C18:1 *trans*-16**	0.57	0.54	0.70	0.69	0.084	0.443
C16:2	4.22	3.80	3.74	3.94	0.219	0.446
C18:2n-6	1311	1085	1053	1179	95.48	0.259
C18:2 cis-9, trans-11	5.68	5.31	5.49	5.40	0.328	0.871
C18:3n-6	3.04	2.72	2.62	3.38	0.338	0.407
C20:2n-6	9.92	8.23	7.86	8.47	0.788	0.306
C20:3n-6	6.52	5.92	5.77	5.84	0.263	0.177
C20:4n-6	53.5	47.6	48.8	47.5	1.904	0.124
C22:4n-6	17.4	15.2	15.7	15.7	0.605	0.084
C22:5n-6	5.87	5.66	5.56	5.72	0.244	0.837
C18:3n-3	70.2	59.0	57.8	65.8	5.756	0.405
C20:5n-3	1.96	1.70	1.82	2.14	0.155	0.229
C22:5n-3	5.32	4.96	5.03	5.32	0.335	0.802
C22:6n-3	1.02	0.97	1.07	1.11	0.106	0.805
C20:3n-9	2.41	2.35	2.33	2.25	0.137	0.882
Other C18:1	12.5	12.17	12.41	12.1	0.434	0.870

Regarding experimental groups, C stands for Control group; CT stands for carrot supplementation, OH stands for oat hay supplementation and CO stand for carrot plus oat hay supplementation; Different superscripts in the same row are associated with significant differences ($P < 0.05$); SEM – Standard error of the mean;
* Co-elution of fatty acids (C18:1 *trans*-6, C18:1 *trans*-7 and C18:1 *trans*-8); ** Co-elution of fatty acids (C18:1 *trans*-16 and C18:*1cis*-14.

Table 4
Detailed fatty acid profile in rabbit's meat (expressed as % of total FA).

	Group				Statistic	
	C	CT	OH	CO	SEM	P
C10:0	0.23	0.25	0.27	0.30	0.030	0.512
C12:0	0.66	0.65	0.64	0.72	0.026	0.208
C13:0	0.02[b]	0.02[b]	0.03[a,b]	0.03[a]	0.001	0.006
C14:0	2.60	2.55	2.49	2.59	0.065	0.607
C15:0	0.50[b]	0.53[a]	0.54[a]	0.54[a]	0.007	0.014
C16:0	24.3	24.1	24.1	24.0	0.286	0.914
C17:0	0.56	0.58	0.58	0.58	0.012	0.640
C18:0	5.62	5.73	5.97	5.78	0.120	0.273
C20:0	0.10	0.11	0.11	0.11	0.002	0.628
C22:0	0.04	0.05	0.04	0.04	0.003	0.275
i-C14:0	0.04	0.04	0.04	0.04	0.001	0.068
i-C15:0	0.06	0.06	0.06	0.06	0.002	0.672
a-C15:0	0.08	0.09	0.09	0.09	0.002	0.167
i-C16:0	0.18	0.19	0.19	0.19	0.006	0.919
i-C17:0	0.04	0.04	0.04	0.04	0.002	0.430
Cyclo-17[1]	0.06	0.07	0.07	0.07	0.004	0.408
C14:1 *cis*-9	0.08	0.07	0.08	0.07	0.010	0.880
C16:1 *cis*-7	0.33	0.33	0.33	0.33	0.007	0.917
C16:1 *cis*-9[2]	1.45	1.40	1.37	1.31	0.104	0.882
C17:1 *cis*-9	0.16	0.16	0.16	0.17	0.007	0.933
C18:1 *cis*-9	25.2	24.8	24.7	24.9	0.233	0.614
C18:1 *cis*-11	1.04	1.05	1.06	1.04	0.015	0.733
C18:1 *cis*-12	0.08	0.10	0.09	0.08	0.008	0.322
C18:1 *cis*-13	0.03	0.03	0.03	0.03	0.002	0.597
C20:1 *cis*-11	0.22	0.22	0.22	0.22	0.006	0.946
C18:1 *trans*-6/−7/−8[3]	0.03	0.03	0.03	0.03	0.002	0.351
C18:1 *trans*-9	0.13[b]	0.14[a,b]	0.15[a]	0.14[b]	0.005	0.007
C18:1 *trans*-10	0.02[a,b]	0.01[b]	0.02[a,b]	0.02[a]	0.001	0.016
C18:1 *trans*-11	0.10	0.11	0.11	0.12	0.007	0.552
C18:1 *trans*-12	0.10	0.11	0.12	0.10	0.007	0.374
C18:1 *trans*-16[4]	0.01[b]	0.01[a,b]	0.02[a]	0.02[a]	0.002	0.005
C16:2n-6	0.10	0.11	0.11	0.10	0.003	0.237
C18:2n-6	30.5	30.5	30.1	30.4	0.329	0.738
C18:2 *cis*-9,*trans*-11	0.14	0.15	0.16	0.14	0.009	0.159
C18:3n-6	0.07	0.07	0.08	0.09	0.005	0.211
C20:2n-6	0.23	0.24	0.21	0.22	0.010	0.491
C20:3n-6	0.16	0.17	0.17	0.17	0.009	0.696
C20:4n-6	1.30	1.39	1.53	1.38	0.105	0.503
C22:4n-6	0.42	0.45	0.49	0.45	0.030	0.501
C22:5n-6	0.14	0.16	0.17	0.16	0.010	0.219
C18:3n-3	1.64	1.67	1.63	1.69	0.018	0.056
C20:5n-3	0.05	0.05	0.05	0.06	0.003	0.192
C22:5n-3	0.13	0.14	0.15	0.15	0.009	0.369
C22:6n-3	0.03	0.03	0.03	0.03	0.002	0.342
C20:3n-9	0.06	0.07	0.07	0.07	0.004	0.610
Other C18:1	0.30	0.33	0.36	0.31	0.016	0.088

Regarding experimental groups, C stands for Control group; CT stands for carrot supplementation, OH stands for oat hay supplementation and CO stand for carrot plus oat hay supplementation; Different superscripts in the same row are associated with significant differences ($P < 0.05$); SEM – Standard error of the mean.

[1] cyclo-17: 11-cyclohexyl-11:0.
[2] Might co-elute with a-C17:0.
[3] Co-elution of fatty acids (C18:1 *trans*-6, C18:1 *trans*-7 and C18:1 *trans*-8).
[4] Might coelute with C18:1*cis*-14.

generally classified as the leanest cut of rabbit meat, with reported FA values ranging from 1.4 to 4.4 g/100 g (Dalle Zotte & Szendro, 2011; Kouba et al., 2008; Rasinska et al., 2018). The values recorded in the present study for the LTL muscle fall within this established range. From a nutritional perspective, the lack of dietary influence on total FA content is advantageous, considering that rabbit meat is commonly acknowledged as a low-fat, high-quality protein source (Dalle Zotte, 2002). Therefore, increased fat content could compromise its nutritional appeal due to higher caloric density, whereas an excessively low lipid level might negatively affect sensory qualities such as tenderness and juiciness, which are closely linked to intramuscular fat (Dalle Zotte, 2002; Gondret et al., 1998).

The fatty acid (FA) composition of rabbit meat was dominated by saturated (SFA) and polyunsaturated fatty acids (PUFA), which averaged 1.32 and 1.29 mg/100 g of meat, corresponding to 35.3 % and 34.7 % of the total FA content, respectively. Monounsaturated fatty acids (MUFA) were less abundant, contributing 1.07 mg/100 g of meat or 28.8 % of the total. Among the SFA, 1.4 % exhibited branched-chain structures, whereas 98.6 % were linear. In the MUFA group, 1.4 % were identified as *trans* isomers, while 98.3 % had the *cis* configuration. Regarding PUFAs, the majority (94.4 %) belonged to the n-6 family, with a smaller proportion (5.4 %) classified as n-3. Statistical tendencies were observed for total PUFA ($P = 0.091$) and total n-6 PUFA ($P = 0.088$) when analyzing the absolute content of key FA groups. Additionally, analysis based on FA proportions revealed a near-significant trend for odd- and branched-chain fatty acids (OBCFA; $P = 0.062$), as well as significant differences in total n-3 PUFA ($P = 0.022$) and the combined sum of OBCFA and biohydrogenation intermediates (referred to as OBCTFA; $P = 0.024$). These differences were exclusively associated with the comparison between the concentrate group (CC) and the oat hay-supplemented group (OH), the experimental groups with most divergent proportion of both NDF and ADF. On the other hand, no other notable trends were observed in the remaining dietary comparisons.

In the evaluation of fatty acid ratios and lipid quality indices, a tendency toward statistical significance was noted for the peroxidability index (PI; $P = 0.095$). A significant difference was identified in the n-6/n-3 PUFA ratio ($P = 0.034$), with higher values recorded in the group receiving only concentrate feed (Group CC), averaging 17.8, compared to 17.1 in the group supplemented with both oat hay and carrot (Group CO). Intermediate ratios were observed in groups fed oat hay (Group OH) or carrot (Group CT) alone, but these did not significantly differ from the extreme values ($P > 0.05$).

The n-6/n-3 ratio serves as an important marker of nutritional balance between these two essential fatty acid families. Although the difference between groups was statistically significant, the actual variation was minor, spanning from 17.8 to 17.1. This suggests limited biological relevance in the context of overall PUFA balance. However, the n-3 PUFA contents in rabbit meat could be increased and the n-6/n-3 ratio decreased by the incorporation of *Medicago sativa* or *Salvia hispanica* in rabbit's diet (Capra et al., 2013; Dal Bosco et al., 2014; Peiretti & Meineri, 2008) or by diet supplementation with linseed (Petracci et al., 2009).

Tables 3 and 4 present the fatty acid (FA) composition of rabbit meat as both absolute values (mg/100 g of meat) and relative percentages (% of total FA). This combined representation provides a more comprehensive understanding of nutritional quality and the extent to which dietary treatments modulate FA profiles. The intramuscular FA composition included nine major fatty acids, each contributing at least 1.0 % of the total FA content, spanning different FA families. These consisted of: linoleic acid (C18:2 n-6; 30.4 %), oleic acid (C18:1 *cis*-9; 24.9 %), palmitic acid (C16:0; 24.1 %), stearic acid (C18:0; 5.8 %), myristic acid (C14:0; 2.6 %), α-linolenic acid (C18:3 n-3; 1.7 %), arachidonic acid (C20:4 n-6; 1.4 %), palmitoleic acid (C16:1 *cis*-9; 1.4 %), and *cis*-vaccenic acid (C18:1 *cis*-11; 1.0 %). Cumulatively, these fatty acids accounted for approximately 93.2 % of the total lipid profile, equivalent to 3.53 g/100 g of meat, and no significant differences on these major FA were detected among dietary treatments ($P > 0.05$). This compositional consistency aligns with earlier studies on rabbit meat (Dal Bosco et al., 2004, 2014; Forrester-Anderson et al., 2006; Kouba et al., 2008; Lazzaroni et al., 2009; Pla, 2008; Polak et al., 2006; Rasinska et al., 2018). Two factors may explain the limited dietary effect of the diets used herein: first, rabbit loin is naturally low in fat and largely composed of structural fatty acids embedded in membrane phospholipids, which are less responsive to dietary shifts and more genetically regulated (Xue, 2016), second, the inclusion levels of carrot and oat hay were deliberately kept low to avoid digestive issues, meaning that the concentrate feed remained the primary source of dietary fatty acids.

When analyzed in terms of absolute concentrations, the detailed fatty acid (FA) profile revealed one statistically significant difference among

dietary groups, specifically for isoC15:0 ($P = 0.039$), and six additional tendencies ($0.05 < P < 0.10$), involving C12:0, C13:0, C14:0, isoC16:0, C18:1 *trans*-12, and C22:4n-6. In relative terms (% of total FA), significant differences were found for C13:0, C15:0, C18:1 *trans*-9, C18:1 *trans*-10, and the co-eluting pair C18:1 *trans*-16 / C18:1 *cis*-14. Furthermore, statistical tendencies were detected for isoC14:0, C18:3 n-3, and the combined group of minor C18:1 isomers.

Overall, regardless of whether results were expressed in absolute or relative terms, most statistically relevant changes occurred in minor fatty acids with limited nutritional impact. However, two noteworthy exceptions emerged. First, rabbits supplemented with oat hay showed a trend toward reduced myristic acid (C14:0) content compared to those fed only concentrate ($P = 0.081$). Second, a trend toward increased α-linolenic acid (C18:3 n-3) proportion was observed in the group receiving both carrot and oat hay ($P = 0.055$), relative to the control.

Both myristic and α-linolenic acids are recognized as major contributors to the FA profile in rabbit meat and are of nutritional importance. Myristic acid has been identified as a hypercholesterolemic SFA, whereas α-linolenic acid plays a key role as an essential n-3 PUFA and a precursor for long-chain n-3 derivatives.

In addition to the primary fatty acids, rabbit meat also contains several odd-numbered straight-chain and methyl-branched-chain fatty acids (OBCFA), which are derived mainly from bacterial lipid membranes (Kaneda, 1991). These compounds have been recognized as bioactive lipids with potential health benefits (Taormina et al., 2020).

Furthermore, C18 biohydrogenation intermediates, such as trans-octadecenoic acids and conjugated linoleic acid (CLA) isomers, were also detected. While these *trans* fatty acids are commonly associated with microbial hydrogenation processes in the rumen of ruminants, their presence in rabbit meat is likely linked to hindgut fermentation and the unique digestive behavior of rabbits. Specifically, rabbits engage in cecotrophy, a process whereby they produce and re-ingest nutrient-rich cecotrophs (soft feces) directly from the anus. This behavior allows the absorption of microbial metabolites synthesized in the caecum (Gidenne et al., 2010), including OBCFA from bacterial membranes and biohydrogenation intermediates formed during the microbial transformation of unsaturated fatty acids in the hindgut (Gómez-Conde et al., 2006; Leiber et al., 2008).

The absolute concentrations of OBCFA and OBCTFA in rabbit meat were not significantly affected by dietary treatment ($P > 0.05$). However, a trend was observed for the relative proportion of OBCFA ($P = 0.062$), and a statistically significant difference was found in the relative abundance of OBCTFA ($P = 0.024$). In both cases, the variations were primarily driven by the comparison between the group receiving only concentrate (Group CC) and the group supplemented solely with oat hay (Group OH). Specifically, rabbits fed oat hay exhibited the highest proportion of OBCTFA (2.36 % of total fatty acids), whereas those receiving only concentrate had the lowest (2.17 %). The presence of OBCTFA reinforces the notion that rabbits are capable of incorporating fatty acids of microbial origin into muscle tissue. Moreover, the observed variation among diets suggests that the inclusion of oat hay and carrot, whether individually or in combination, may beneficially modulate gut microbiota activity. This effect is likely attributed to the increased intake of neutral detergent fiber (NDF), which was higher in the supplemented diets compared to the control concentrate-based diet (Quaresma et al., 2023).

In the assessment of fatty acid ratios and lipid quality indices, dietary treatments exerted a statistically significant effect solely on the n-6/n-3 ratio ($P = 0.048$), while no significant differences were detected in the remaining FA ratios or in any of the lipid quality indices ($P > 0.05$).

The average hypocholesterolemic/hypercholesterolemic (h/H) ratio was 2.37, indicating that beneficial hypocholesterolemic fatty acids were present in more than twice the proportion of their hypercholesterolemic counterparts. The mean polyunsaturated/saturated fatty acid (P/S) ratio was 0.99, suggesting a near-equilibrium between PUFA and SFA contents. Atherogenic (AI) and thrombogenic (TI) indices were consistently low across treatments, averaging 0.55 and 0.89, respectively.

Despite these favorable indices, the n-6/n-3 ratio remained notably unbalanced, with values ranging from 17.1 to 17.8, reflecting a disproportionate prevalence of n-6 PUFA relative to n-3 PUFA in rabbit meat lipid composition.

3.2. Total cholesterol and vitamin E contents and tocochromanols profile

Table 5 summarizes the concentrations of total cholesterol, total vitamin E, and the main vitamin E homologues (α- and γ-tocopherols) in rabbit meat. The α-tocopherol was the predominant tocochromanol, accounting for 95.3 % to 97.4 % of the total vitamin E content.

The dietary treatments had a statistically significant effect on the concentrations of total cholesterol ($P = 0.003$), total vitamin E ($P < 0.001$), α-tocopherol ($P < 0.001$), and γ-tocopherol ($P = 0.003$). Rabbits receiving concentrate as the sole diet (Group CC) exhibited the highest cholesterol levels (46.2 mg/100 g of meat), differing significantly from animals receiving oat hay, either alone or combined with carrot, which averaged 33.9 mg/100 g. The group supplemented solely with carrot showed intermediate values (38.1 mg/100 g), with no significant differences from the other groups ($P > 0.05$).

The total cholesterol content observed in group CT aligns with the muscle-specific profile, Earlier studies have reported lower cholesterol levels in the *longissimus lumborum* muscle (approximately 45 mg/100 g) compared to whole-carcass values, which typically range from 59 to 68 mg/100 g (Combes, 2004; Polak et al., 2006).

Meat from rabbits fed exclusively with concentrate showed the highest levels of total vitamin E (10.5 μg/g), α-tocopherol (10.2 μg/g), and γ-tocopherol (0.27 μg/g). Significant decreases in total vitamin E content were observed in meat from rabbits supplemented with oat hay, either alone (resulting in a 56.9 % reduction compared to the concentrate group), or combined with carrot (which led to a 55.7 % decrease relative to the concentrate group). These reductions reflected declines in both α- and γ-tocopherol concentrations. Rabbits receiving only carrot supplementation had intermediate values for total vitamin E and α-tocopherol (7.75 and 7.50 μg/g, respectively), differing significantly ($P < 0.001$) from the other groups.

The concentrate feed serves as the primary source of both cholesterol and vitamin E in the diet of rabbits. Cholesterol is majorly supplied by lard, a major component of the concentrate feed, while α-tocopherol is supplied through the mineral-vitamin premix. Consequently, a decrease in the intake of concentrate feed leads to lower bioavailability of these compounds. However, an additional factor may explain the greater reduction in α-tocopherol relatively to total cholesterol levels in meat. Despite similar reductions in concentrate DM consumption between the groups supplemented solely with carrot (CT) or oat hay (OH) (Quaresma et al., 2023), the decline in both molecules was notably more marked in the group receiving oat hay supplementation.

Oat and oat hay are both rich in β-glucans (Fraser & Wilkie, 1971; Liu & Mahmood, 2015), compounds often linked to cholesterol-lowering effects (Mushtaq et al., 2014). The mechanisms behind the

Table 5
Total cholesterol, total vitamin E and tocochromanols in rabbit's meat.

	Group				Statistic	
	CC	CT	OH	CO	SEM	P
Cholesterol[1]	46.16^{a}	38.08^{a,b}	34.26^{b}	33.60^{b}	0.04	<0.001
Vitamin E[2]	10.49^{a}	7.75^{b}	4.49^{c}	4.66^{c}	0.73	<0.001
α-tocopherol	10.22^{a}	7.50^{b}	4.28^{c}	4.47^{c}	0.72	<0.001
γ-tocopherol	0.27^{a}	0.25^{a,b}	0.21^{b,c}	0.19^{c}	0.01	<0.001

Different superscripts in the same row are associated with significant differences ($P < 0.05$); SEM – Standard error of the mean.
[1] Expressed as mg/100 g of fresh meat.
[2] Expressed as μg/g of fresh meat.

hypocholesterolemic action of β-glucans are not entirely clear but are thought to involve: 1) increased digesta viscosity, which reduces the absorption of lipids such as triglycerides and cholesterol from the intestine; and 2) interference with the enterohepatic recycling of bile acids, leading to increased fecal excretion of bile acids along with cholesterol. This results in a lower intestinal uptake of cholesterol and a higher requirement of cholesterol to synthesize bile acids, ultimately decreasing plasma cholesterol levels and cholesterol deposition in tissues (Mushtaq et al., 2014; Sima et al., 2018).

In contrast, the cholesterol-lowering effect of carrot was milder. However, carrot leaves contain high levels of crude fiber, which can bind cholesterol and facilitate its elimination (Puspani et al., 2020). Additionally, the carrot root is rich in pectin, a soluble fiber that increases digesta viscosity and may reduce nutrient bioavailability, including cholesterol (Capuano, 2017; Kale et al., 2013; Theuwissen & Mensink, 2008).

Dietary supplementation with carrot and/or oat hay significantly decreased meat vitamin E levels, with a more pronounced reduction observed in groups supplemented with oat hay alone or combined with carrot (58.1 % and 56.3 % decrease, respectively), while carrot supplementation alone caused a moderate decrease of 26.1 %. Although cholesterol and α-tocopherol are both lipophilic molecules, the α-tocopherol's chromanol ring increases its polarity relative to cholesterol, potentially strengthening its interaction with digesta components. This may hamper its incorporation into micelles and absorption in the intestine, possibly explaining the greater reduction in α-tocopherol levels relatively to total cholesterol, as was observed herein. Lower vitamin E content in meat could increase susceptibility to oxidative degradation, negatively affecting meat color (via myoglobin oxidation), sensory qualities (through development of off-flavors), and nutritional value (Nakyinsige et al., 2015; Tang et al., 2005).

Beyond differences associated with diet, the rabbit meat in analysis can be classified as extra lean, since 100 g of rabbit meat (LTL muscle) contains less than 95 mg of cholesterol, less than 5 g of total fat and less than 2 g of saturated fat (U.S. FDA, 2017).

Beyond the results reported herein, a comparative assessment of rabbit meat with other lean meats, namely broiler breast and goat kid loin, offers additional insight into its nutritional profile. Rabbit meat exhibits a higher total lipid content but a lower total cholesterol concentration when compared with both broiler breast and goat kid loin (Ponte et al., 2008; Quaresma et al., 2016). This compositional pattern may be considered advantageous, as increased intramuscular lipid content is positively associated with improved tenderness and flavor attributes, whereas dietary cholesterol is widely recognized for its adverse implications for cardiovascular health. Analysis of the fatty acid composition further revealed that rabbit meat contains slightly greater proportions of polyunsaturated fatty acids (PUFA) and lower proportions of monounsaturated fatty acids (MUFA), while saturated fatty acid (SFA) levels remain comparable across species. Moreover, the α-tocopherol concentration in rabbit meat ranged from 5.75 to 10.5 μg/g, whereas the comparator meats typically exhibited values closer to the lower limit of this range, suggesting a potentially superior contribution to the antioxidant status of rabbit meat.

4. Conclusions

Supplementation with oat hay and carrot, either individually or combined, produced modest changes in the total fatty acid (FA) content and FA profile of rabbit meat, with the combined supplementation notably increasing total n-3 PUFAs and reducing the n-6/n-3 PUFA ratio. Oat hay alone decreased total cholesterol and vitamin E contents but increased odd- and branched-chain fatty acids (OBCFAs). These feeding strategies not only reduce feed costs but also yield minor yet nutritionally beneficial modifications in rabbit meat lipid composition, supporting the inclusion of oat hay and dehydrated carrot in concentrate formulations, potentially with additional vitamin E. Overall, the findings demonstrate the feasibility of using alternative feed ingredients to lower production costs while improving the nutritional quality of rabbit meat for human consumption.

CRediT authorship contribution statement

Mário A.G. Quaresma: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Gonçalo Pereira:** Investigation, Formal analysis, Conceptualization. **Maria Leonor Nunes:** Writing – review & editing, Formal analysis. **Helena Gonçalves:** Investigation. **Rui J.B. Bessa:** Investigation, Formal analysis. **Cristina Roseiro:** Supervision, Resources, Investigation, Data curation. **Susana P. Alves:** Writing – review & editing, Data curation.

Author agreement

As the corresponding author of this manuscript, I am in the condition to certify that all authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Author statement

All authors have contributed significantly to the manuscript and agree to its submission to Meat Science. The specific contributions of each author are listed below accordingly the Credit taxonomy.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used the ChatGPT to improve the language and grammar accuracy, but the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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