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# Nutritional Composition of Commercially Sourced Meat from Two Anatomical Locations Under Regenerative and Intensive Production Systems

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## Abstract

Regenerative agriculture approaches in livestock production may help produce animal protein that aligns with increasingly demanding sustainability criteria. This study compared commercially sourced beef from regenerative farming systems (RFS; $n$ = 10; *Longissimus lumborum*, $n$ = 5; *Splenius capitis*, $n$ = 5) and intensive systems (IS; $n$ = 6; *Longissimus lumborum*, $n$ = 3; *Splenius capitis*, $n$ = 3), evaluating the effects of production system (PS), muscle type (MT), and their interaction (MT × PS) on nutritional traits. IS chuck had higher polyunsaturated fatty acids (PUFA) and n-6 PUFA family (n-6 PUFA) contents ($p < 0.05$; 10.24 and 9.15 g fatty acids (FA)/100 g total FA, respectively), driven by C18:2 *cis*-9, *trans*-11, C20:4 n-6, and C18:2 n-6 contents. Consequently, IS chuck had a higher polyunsaturated FA and saturated FA ratio (P/S), peroxidability index (PI), n-6 PUFA family and n-3 PUFA family ratio (n-6/n-3), and hypocholesterolemic/hypercholesterolemic ratio (h/H) values ($p < 0.05$; 0.13, 23.87, 9.33 and 0.32, respectively). By comparison, RFS chuck had the highest n-3 PUFA content ($p < 0.05$; 1.28 g FA/100 g total FA), primarily due to its higher C18:3 n-3 content, resulting in a lower n-6/n-3 ratio (3.95). RFS meat showed higher vitamin E and $\alpha$-tocopherol (0.58 and 0.56 mg/100 g of meat, respectively), exceeding the $\geq$0.30 mg/100 g threshold proposed to limit lipid oxidation, unlike IS meat.

**Keywords:** regenerative farming systems; meat quality; nutritional composition



Academic Editor: Paolo Polidori

Received: 29 December 2025
Revised: 24 February 2026
Accepted: 4 March 2026
Published: 7 March 2026



## 1. Introduction

Livestock production is facing growing consumer scrutiny due to concerns about environmental impacts, animal welfare, and public health [1,2]. As a result, interest has

grown in alternative livestock systems, including regenerative farming systems (RFS), since regenerative agriculture principles applied to livestock production may improve farms' environmental performance while maintaining animal welfare standards [3,4]. Practices such as rotational or adaptive grazing, in which stocking rates and grazing periods are adjusted according to pasture availability and recovery [5,6], can reduce dependence on concentrates and increase the contribution of pasture-based feeding [7]. When combined with crop–livestock integration, these strategies can strengthen nutrient cycling and reduce reliance on external inputs [4,8]. Ultimately, regenerative approaches may help produce animal protein that meets increasingly demanding sustainability criteria and contributes to the resilience of agricultural systems [7].

In contrast, intensive systems (IS) are often highly efficient but may rely more heavily on purchased feeds and other external inputs. They can also be associated with adverse impacts on soil health, biodiversity, and greenhouse gas emissions, and, in some contexts, may compromise animal welfare [7–9].

Because diet strongly influences meat composition, variation in pasture intake and associated antioxidant intake may affect the nutritional and physicochemical properties of beef. Pasture-based diets, in particular, have been linked to a more favourable fatty acid (FA) profile (e.g., higher n-3 polyunsaturated FA (PUFA) and a lower n-6/n-3 ratio) and greater concentrations of antioxidants such as vitamin E, which may in turn influence colour stability [10–13]. However, beef fat content varies with several factors, including species, feeding regime, and the specific meat cut [10,14,15]. Therefore, transitioning from intensive production to regenerative farming systems could lead to measurable differences in meat quality.

Beef remains a nutrient-dense food [16], providing high-biological-value protein and a range of essential micronutrients, including fat- and water-soluble vitamins (A, D, B9, and B12) and minerals such as iron, zinc, and selenium [14]. Additionally, its lipid fraction contributes to energy intake and facilitates the absorption of fat-soluble vitamins [10]. The absence of naturally occurring B12 vitamin in plant-based foods and the reduced efficiency of β-carotene (provitamin A) conversion to vitamin A further reinforces the relevance of beef as a source of these nutrients in the human diet [14,15]. Nevertheless, the total lipid content of beef, and particularly its FA profile, has been a matter of concern, since it is a significant source of saturated FA in the human diet. A higher consumption of total fat, and especially saturated FA, has been associated with an increased risk of cardiovascular disease [10,16,17].

This study aims to compare commercially sourced beef from RFS and IS, focusing on how production systems and muscle types jointly shape technological and nutritional quality. Unlike many studies based on experimentally managed animals under controlled conditions, our work uses market-available beef and evaluates two cuts, providing a consumer-relevant perspective. Specifically, we assessed proximate composition, pH, colour, vitamin E, and a detailed FA profile. We hypothesised that beef from RFS would show significant differences across multiple quality parameters, reflecting the influence of the production system.

## 2. Materials and Methods

### *2.1. Sampling and Sample Processing*

Commercial beef samples were obtained through VividFarms (Santarém, Portugal), a company specialising in meat processing and packaging. Samples were sourced directly from the commercial supply chain and, consequently, farm-level variables such as pasture botanical composition, stocking rate, grazing rotation metrics, and seasonal forage composition were not directly measured and could not be controlled. The samples rep-

resented two production systems: a regenerative farming system based in Portugal (RFS; sirloin—*Longissimus lumborum*, $n$ = 5; chuck—*Splenius capitis*, $n$ = 5) and an intensive system (IS) from Poland (sirloin—*Longissimus lumborum*, $n$ = 3; chuck—*Splenius capitis*, $n$ = 3). Unequal sample numbers reflected product availability constraints from the supplier during the sampling period. Each sample consisted of a ~200 g single steak (approximately 2 cm thick), and samples were transported from the supplier in a thermal bag under refrigerated conditions, with temperature maintained between 3° and 5 °C until arrival at the INIAV facilities. At 48 h post-mortem, pH and colour measurements were performed. Subsequently, the samples were homogenised using a food processor (Moulinex, Alençon, France), vacuum-packed, and stored at −80 °C until further analysis.

### 2.2. pH Measurement

The pH of each sample was measured using a digital pH meter (Crison 507, Barcelona, Spain) fitted with a penetration electrode (model 25-21, Crison).

### 2.3. Colour Measurement

Colour analysis of each sample was performed in quintuplicate under controlled temperature conditions (20 °C ± 1 °C), using a Konica Minolta CR-400 reflectance colourimeter (Konica Minolta Sensing Inc., Osaka, Japan) operated through the SpectraMagic NX software (version 3.40). Measurements were obtained with a D65 illuminant and a 2° standard observer. Before analysis, the instrument was calibrated with a white reference tile (Y = −84.3; X = −0.3178; Y = −0.3342). The CIELab coordinates—lightness (L*), red/green (a*), and yellow/blue (b*) were recorded for each measurement. Chroma (C*) and hue angle (h°) were calculated.

### 2.4. Proximate Composition Determination

Homogenised meat samples were used to determine their proximate composition following AOAC methods, specifically moisture (950.46), total protein (981.10), total fat (991.36), and ash (920.153).

The total carbohydrate content (TCC) was estimated by difference, using the following equation [18]: TCC (g/100 g) = 100 − moisture content − total protein content − total fat content − ash content.

The energy content was calculated according to the Atwater specific factor system [18] as follows: Energy (kcal/100 g) = (protein content × 4) + (carbohydrate content × 4) + (fat content × 9).

### 2.5. Determination of Tocopherol Profile

The tocopherol profile content was analysed according to the methodology previously described [19]. Briefly, 5 g of each sample was subject to saponification in a water bath at 80 °C for 15 min with 20 mL of saponification solution (11% $w/v$ potassium hydroxide (KOH) in a mixture of 55% $v/v$ absolute ethanol and 45% $v/v$ distilled water) and 0.6 g of ascorbic acid. After this time, the tubes were cooled with tap water for 1 min. Then, 6 mL of distilled water and 12 mL of n-hexane with butylated hydroxytoluene (BHT; 25 µg/mL) were added, vortexed for 2 min, and the tubes were centrifuged at 1500× $g$ for 5 min. The top layer was filtered over anhydrous sodium sulphate and filtered again with a 0.45-µm hydrophobic acrodisc (Filter lab, Barcelona, Spain). The filtrate was injected into a Waters High-Performance Liquid Chromatography (HPLC) system. Tocopherols were detected using a Waters 474 fluorescence detector (excitation 295 nm, emission 325 nm). Separations were carried out on a normal-phase silica column (Waters Silica, 125 mm × 4.6 mm i.d., 5 µm; Waters, Milford, MA, USA), using 1% $v/v$ isopropanol in n-hexane as solvent at a flow rate of 1.0 mL/min, with an injection volume of 100 µL. Tocopherols were identified

by comparing retention times with those of authentic standards, and concentrations were quantified using the external standard method.

### 2.6. Determination of Fatty Acid (FA) Profile

#### 2.6.1. Extraction of Intramuscular Fat

Intramuscular fat was extracted after removal of visible adipose and connective tissue, following the method described by Folch et al. (1957) [20] using a dichloromethane/methanol solvent system instead of chloroform, a modification previously validated for lipid extraction and FA profiling [21]. Briefly, 3 g of each sample wasmixed with 20 mL of a dichloromethane/methanol solution (2:1, $v/v$) and homogenised using a Polytron PT 3000 homogeniser (Kinematica, Luzern, Switzerland) at 10,000 rpm for 1 min. The homogenate was vacuum filtered through a glass microfiber filter (Whatman GF/C) mounted on a Büchner funnel. The filtrate was transferred to a separatory funnel, and 5 mL of an aqueous NaCl solution (0.73%, $w/v$) was added. After vigorous shaking, the mixture was allowed to stand at room temperature for 12 h to promote phase separation. The lower organic phase was collected, and the solvent was removed using a rotary evaporator (Laborota 4000, Heidolph Instruments, Schwabach, Germany) at 45 °C. The resulting lipid residue was dried in an oven (Memmert U10, Schwabach, Germany) at 103 °C to constant weight for gravimetric determination of total lipid.

#### 2.6.2. Methylation and Chromatographic Analysis

FA from intramuscular fat were derivatised to FA methyl esters (FAMEs) using a rapid methylation procedure with 2 N methanolic potassium hydroxide, in accordance with NP EN ISO 5509:2003 [22]. The resulting FAMEs were separated, identified, and quantified by gas chromatography (GC) with flame ionisation detection (FID) on a Trace GC 2000 series chromatograph (Thermo Quest, CE Instruments, Rodano, Milan, Italy) equipped with a DB-23 capillary column (50% cyanopropyl methylpolysiloxane, 60 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature programme was initiated at 195 °C and increased to 210 °C at 5 °C/min, with a final hold of 150 min. Injector and detector temperatures were set at 220 °C and 280 °C, respectively. Helium was used as the carrier gas at 70 kPa, with a split ratio of 1:40, and 0.3 µL of sample was injected. FA were identified by comparing their retention time with those of commercial FAME standards (Supelco Inc., Bellefont, PA, USA).

#### 2.6.3. FA Ratios and Lipid Quality Indices

The nutritional FA ratios polyunsaturated/saturated (P/S) and n-6/n-3 were calculated using the equation previously established by the British Department of Health (1994) [23]:

$$P/S = [(C18{:}2\ n\text{-}6 + C18{:}3\ n\text{-}3)/(C14{:}0 + C16{:}0 + C18{:}0)]$$

$$n\text{-}6/n\text{-}3 = [(\sum n\text{-}6\ PUFA)/(\sum n\text{-}3\ PUFA)]$$

The indices of atherogenicity (AI) and thrombogenicity (TI) were estimated as proposed by Ulbricht and Southgate (1991) [24]:

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

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/[(0.5 \times \sum MUFA + 0.5 \times (n-6) + 3 \times (n-3) + (n-3)/(n-6))]$$

The peroxidability index (PI) was calculated according to the equation previously proposed by Arakawa and Sagai (1986) [25]:

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

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated using the equation previously proposed by Santos-Silva et al. (2002) [26]:

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

### *2.7. Statistical Analysis*

The statistical analysis was carried out using STATISTICA software (version 12; StatSoft Inc., Tulsa, OK, USA). A two-way analysis of variance (ANOVA) was used to evaluate the effects of muscle type (MT), production system (PS), and their interaction (MT × PS) on each response variable. Muscle type and production system were treated as fixed factors. Each response variable was analysed independently using all available observations. When a significant interaction was detected ($p < 0.05$), results were interpreted using simple effects, and comparisons were performed across MT × PS combinations. In the absence of a significant interaction, the main effects were interpreted independently. Post hoc comparisons were conducted using Fisher's least significant difference (LSD) test and applied only when the corresponding ANOVA effect was significant ($p < 0.05$). Tables report MT × PS interaction means (mean ± standard error); different superscript letters denote significant differences between means (LSD, $p < 0.05$). When the MT × PS interaction was not significant ($p > 0.05$), superscripts refer to main-effect comparisons only, and values presented in the text correspond to the model-estimated marginal means (least-squares means) for the significant main effects, averaged across the other factor.

## 3. Results and Discussion

### *3.1. Proximate Composition*

Energy, moisture, fat, and carbohydrate contents were significantly affected by the MT × PS interaction ($p < 0.05$; Table 1), whereas the interaction was not significant ($p > 0.05$) for protein or ash content.

**Table 1.** Proximate composition (expressed as g/100 g of meat) of beef sirloin (*Longissimus lumborum*) and chuck (*Splenius capitis*) from different production systems (intensive system, IS; regenerative farming systems, RFS).

| | Sirloin | | Chuck | | *p* | | |
|---|---|---|---|---|---|---|---|
| | **IS** | **RFS** | **IS** | **RFS** | **MT** | **PS** | **MT × PS** |
| Energy (kcal/100 g of meat) | $110.86^{b} \pm 2.92$ | $143.95^{a} \pm 10.56$ | $110.03^{b} \pm 24.16$ | $112.11^{b} \pm 5.92$ | 0.024 | 0.028 | 0.030 |
| Moisture | $74.21^{a} \pm 0.4$ | $71.52^{b} \pm 0.56$ | $74.63^{a} \pm 3.03$ | $75.95^{a} \pm 0.51$ | 0.006 | 0.401 | 0.016 |
| Protein | $21.02^{a} \pm 0.32$ | $18.72^{b} \pm 0.25$ | $20.59^{a} \pm 1.32$ | $17.93^{b} \pm 0.48$ | 0.140 | <0.001 | 0.788 |
| Fat | $2.47^{b} \pm 0.27$ | $6.80^{a} \pm 1.82$ | $3.50^{b} \pm 1.82$ | $4.01^{b} \pm 0.87$ | 0.245 | 0.006 | 0.021 |
| Carbohydrates | $1.13^{b} \pm 0.29$ | $3.26^{a} \pm 0.56$ | $1.33^{b} \pm 0.19$ | $1.53^{b} \pm 0.35$ | 0.016 | 0.002 | 0.005 |
| Ash | $1.17^{a} \pm 0.09$ | $0.99^{b} \pm 0.04$ | $1.16^{a} \pm 0.06$ | $1.04^{b} \pm 0.02$ | 0.392 | <0.001 | 0.282 |

Values are expressed as mean ± standard error (SE). Different superscript letters indicate significant differences between means according to Fisher's LSD test ($p < 0.05$).

Energy density is mainly determined by a food's macronutrient and moisture content. Fat contributes most strongly because it provides 9 kcal/g compared with 4 kcal/g for protein and carbohydrates. Consequently, foods higher in fat tend to be more energy-dense, while a higher moisture content reduces energy density, since water adds weight without

providing energy [27]. In line with this, sirloin from RFS showed higher energy values (144 kcal/100 g of meat), fat content (6.8 g/100 g of meat), and carbohydrate content (3.3 g/100 g of meat) than sirloin from the IS and beef chuck, regardless of production system. Conversely, moisture content was higher in IS sirloin and chuck, as well as in RFS chuck (mean 74.9 g/100 g meat), compared with RFS sirloin. This inverse pattern between energy (driven mainly by fat, and to a lesser extent carbohydrate) and moisture across the MT × PS interactions is consistent with the well-established dilution effect, whereby increased lipid deposition raises energy density while concomitantly reducing moisture content [27].

The higher fat content observed in RFS sirloin may reflect differences in finishing strategy, animal maturity at slaughter, or energy intake from pasture-based diets, and may also be influenced by unmeasured animal-level factors. However, because samples were sourced through the commercial supply chain and detailed metadata were not available, these explanations cannot be conclusively established based on the available information and should be evaluated in controlled studies with comprehensive animal- and farm-level information.

A significant main effect of production system was observed for protein and ash contents ($p < 0.05$), whereas muscle type had no significant effect ($p > 0.05$) on these variables. Meat from the IS exhibited significantly higher protein and ash content (20.8 and 1.2 g/100 g of meat, respectively) than meat from RFS (18.3 and 1.0 g/100 g of meat, respectively). We hypothesise that the higher protein content observed in IS may be associated with differences in growth rate, carcass composition, and feeding regimes [28]. Nevertheless, the lack of detailed information on animal production conditions underlying these systems limits a more precise interpretation of these results.

### 3.2. pH and Colour

The absence of a significant MT × PS interaction effect for pH ($p > 0.05$) indicates that the factors tested did not combine synergistically to influence pH; rather, pH showed a significant main effect of production system ($p < 0.05$). Based on the model-estimated marginal means (averaged across muscle type), meat from the IS had a higher pH than meat from RFS (5.58 vs. 5.48; $p < 0.05$, Table 2). Although differences between groups were detected, all pH values remained within the normal range reported for beef.

**Table 2.** pH values and colourimetric parameters of beef sirloin (*Longissimus lumborum*) and chuck (*Splenius capitis*) from different production systems (intensive system, IS; regenerative farming systems, RFS).

| | Sirloin | | Chuck | | *p* | | |
|---|---|---|---|---|---|---|---|
| | **IS** | **RFS** | **IS** | **RFS** | **MT** | **PS** | **MT × PS** |
| pH | $5.57^{a} \pm 0.05$ | $5.49^{b} \pm 0.03$ | $5.59^{a} \pm 0.03$ | $5.47^{b} \pm 0.06$ | 0.789 | 0.002 | 0.480 |
| L* | $37.91^{a} \pm 0.55$ | $35.09^{b} \pm 0.43$ | $32.75^{c} \pm 1.33$ | $32.82^{c} \pm 0.91$ | <0.001 | 0.007 | 0.005 |
| a* | $17.26^{bc} \pm 0.47$ | $16.04^{c} \pm 0.62$ | $17.44^{b} \pm 0.65$ | $20.13^{a} \pm 1.24$ | <0.001 | 0.127 | 0.001 |
| b* | $12.75^{ab} \pm 0.06$ | $13.22^{a} \pm 0.17$ | $12.08^{b} \pm 0.52$ | $10.47^{c} \pm 1.10$ | <0.001 | 0.132 | 0.012 |
| C* | $21.50^{b} \pm 0.44$ | $20.80^{b} \pm 0.46$ | $21.26^{b} \pm 0.73$ | $22.74^{a} \pm 0.68$ | 0.017 | 0.229 | 0.004 |
| h° | $36.43^{bc} \pm 0.69$ | $39.53^{a} \pm 1.24$ | $34.70^{c} \pm 1.03$ | $27.56^{d} \pm 3.80$ | <0.001 | 0.123 | 0.001 |

Values are expressed as mean ± standard error (SE). Different superscript letters indicate significant differences between means according to Fisher's LSD test ($p < 0.05$).

Meat colour strongly affects consumer acceptance and is mainly determined by myoglobin, but it is also influenced by factors such as diet, age, and physical activity [29]. The MT × PS interaction significantly influenced all colourimetric parameters ($p < 0.05$; Table 2), indicating that the influence of production system on colour differed between muscles. The IS sirloin exhibited the highest lightness (L* = 37.9; $p < 0.05$). However, because IS

sirloin also showed a higher pH than RFS sirloin, this difference cannot be attributed to more extensive post-mortem acidification. Moreover, if lightness differences were mainly driven by pH decline, a comparable increase in L* would also be expected in the IS chuck, which was not observed. Overall, these findings suggest that muscle type is a key driver of lightness differences.

Chuck RFS showed the highest ($p < 0.05$) redness (a*) and chroma (C*) values (20.13 and 22.7, respectively), indicating a redder and more saturated appearance in this cut. A plausible explanation is the higher total vitamin E content of RFS meat (see results below). Vitamin E is known to delay oxymyoglobin oxidation [30], thereby enhancing oxidative stability and supporting the maintenance of a more vivid red colour, as reported elsewhere [31,32].

Furthermore, sirloin, regardless of the production system, presented higher yellowness (b*) values (averaging 13.0), which could suggest greater fat content. However, this explanation is only supported for RFS sirloin, where higher fat content was detected. Therefore, other factors likely contribute to the elevated b* values, such as muscle differences. This is consistent with the F statistics, which indicate that muscle type explains more of the variation in b* (F = 23.9) than the production system, whose main effect was weak (F = 2.61).

The highest hue angle (h°) in RFS sirloin indicates a shift in colour tone relative to other meat types. Consistent with the remaining colour parameters, muscle type also explained more of the variation in this attribute (h°: F = 31.63) than the production system (h°: F = 2.75).

Overall, the colour results indicate that production system effects on colour were muscle-dependent, whereas differences between muscle types represented a stronger source of variation in colour attributes.

### *3.3. Lipid Profile*

#### 3.3.1. Fatty Acids (FA)

The MT × PS interaction was not significant ($p > 0.05$) for all fatty acid (FA) partial sums, FA ratios, and lipid quality indexes (Table 3). Instead, these variables were mainly influenced by muscle type, production system or both ($p < 0.05$). An exception was the saturated FA (SFA), which were not significantly affected by muscle type, production system, or their interaction ($p > 0.05$).

**Table 3.** Fatty acid (FA) partial sums (expressed as g of FA/100 g of total FA), FA ratios, and lipid quality indexes of beef sirloin (*Longissimus lumborum*) and chuck (*Splenius capitis*) from different production systems (intensive system, IS; regenerative farming systems, RFS).

| | Sirloin | | Chuck | | *p* | | |
|---|---|---|---|---|---|---|---|
| | **IS** | **RFS** | **IS** | **RFS** | **MT** | **PS** | **MT × PS** |
| SFA [1] | 48.45 ± 2.76 | 54.6 ± 2.14 | 50.95 ± 2.76 | 50.35 ± 2.14 | 0.730 | 0.283 | 0.196 |
| MUFA [2] | 42.81 [a] ± 2.42 | 36.86 [b] ± 1.87 | 43.03 [a] ± 2.42 | 38.26 [b] ± 1.87 | 0.716 | 0.029 | 0.791 |
| PUFA [3] | 6.19 [bc] ± 1.03 | 3.85 [c] ± 0.8 | 10.24 [a] ± 1.03 | 6.35 [b] ± 0.8 | 0.004 | 0.006 | 0.417 |
| n-6 PUFA [4] | 5.48 [b] ± 0.94 | 2.98 [b] ± 0.72 | 9.15 [a] ± 0.94 | 5.07 [b] ± 0.72 | 0.005 | 0.002 | 0.365 |
| n-3 PUFA [5] | 0.71 [b] ± 0.15 | 0.88 [b] ± 0.12 | 1.1 [ab] ± 0.15 | 1.28 [a] ± 0.12 | 0.012 | 0.220 | 0.938 |
| P/S [6] | 0.1 [ab] ± 0.02 | 0.06 [b] ± 0.01 | 0.13 [a] ± 0.02 | 0.11 [a] ± 0.01 | 0.035 | 0.070 | 0.479 |
| n-6/n-3 [7] | 7.78 [a] ± 0.83 | 3.41 [b] ± 0.64 | 9.33 [a] ± 0.83 | 3.95 [b] ± 0.64 | 0.183 | <0.001 | 0.509 |
| h/H [8] | 0.19 [b] ± 0.04 | 0.09 [b] ± 0.03 | 0.32 [a] ± 0.04 | 0.17 [b] ± 0.03 | 0.006 | 0.003 | 0.504 |

**Table 3.** *Cont.*

| | Sirloin | | Chuck | | *p* | | |
|---|---|---|---|---|---|---|---|
| | **IS** | **RFS** | **IS** | **RFS** | **MT** | **PS** | **MT × PS** |
| AI [9] | 0.84 [b] ± 0.14 | 1.41 [a] ± 0.11 | 0.76 [b] ± 0.14 | 1.11 [ab] ± 0.11 | 0.160 | 0.003 | 0.386 |
| TI [10] | 1.8 [b] ± 0.15 | 2.26 [a] ± 0.12 | 1.62 [b] ± 0.15 | 1.83 [b] ± 0.12 | 0.044 | 0.029 | 0.375 |
| PI [11] | 12.34 [b] ± 1.97 | 7.58 [b] ± 1.53 | 23.87 [a] ± 1.97 | 11.88 [b] ± 1.53 | 0.001 | <0.001 | 0.063 |

Values are expressed as mean ± standard error (SE). Different superscript letters indicate significant differences between means according to Fisher's LSD test ($p < 0.05$). [1] Sum of saturated FA; [2] sum of monounsaturated FA; [3] sum of polyunsaturated FA; [4] n-6 PUFA family; [5] n-3 PUFA family; [6] polyunsaturated FA and saturated FA ratio; [7] n-6 PUFA family and n-3 PUFA family ratio; [8] hypocholesterolemic/hypercholesterolemic ratio; [9] atherogenicity index; [10] thrombogenicity index; [11] peroxidability index.

At the individual FA level, a significant MT × PS interaction ($p < 0.05$) was observed only for iC17:0, C20:0, C18:2 n-6, C20:4 n-6, and C22:4 n-6 (Table 4). Most other FA were mainly influenced by the production system ($p < 0.05$). Exceptions were C18:2 *cis*-9, *trans*-11 and C18:3 n-3, which were influenced by muscle type ($p < 0.05$). In contrast, C14:1 *cis*-9, C16:1 *cis*-9, C17:1 *cis*-9, and C22:5 n-3 were not significantly affected by muscle type, production system, or their interaction ($p > 0.05$), averaging 1.16, 4.04, 0.78, and 0.28 g FA/100 g total FA, respectively.

**Table 4.** Fatty acid (FA) profile (expressed as g of FA/100 g of total FA) of beef sirloin (*Longissimus lumborum*) and chuck (*Splenius capitis*) from different production systems (intensive system, IS; regenerative farming systems, RFS).

| | Sirloin | | Chuck | | *p* | | |
|---|---|---|---|---|---|---|---|
| | **IS** | **RFS** | **IS** | **RFS** | **MT** | **PS** | **MT × PS** |
| C14:0 | 3.18 [b] ± 0.33 | 5.45 [a] ± 1.17 | 3.13 [b] ± 0.71 | 5.07 [a] ± 0.77 | 0.638 | 0.001 | 0.721 |
| C15:0 | 0.38 [b] ± 0.06 | 1.13 [a] ± 0.24 | 0.63 [b] ± 0.2 | 1.14 [a] ± 0.17 | 0.338 | <0.001 | 0.153 |
| C16:0 | 27.17 [b] ± 1.28 | 32.20 [a] ± 1.05 | 27.22 [b] ± 1.28 | 32.23 [a] ± 0.91 | 0.975 | 0.003 | 0.989 |
| iC17:0 | 0.49 [d] ± 0.03 | 0.80 [a] ± 0.05 | 0.58 [c] ± 0.06 | 0.69 [b] ± 0.04 | 0.546 | <0.001 | 0.001 |
| C17:0 | 0.84 [b] ± 0.07 | 1.34 [a] ± 0.06 | 0.92 [b] ± 0.08 | 1.35 [a] ± 0.05 | 0.477 | <0.001 | 0.591 |
| C18:0 | 14.4 [a] ± 1.07 | 10.46 [b] ± 1.09 | 16.53 [a] ± 3.23 | 11.66 [b] ± 1.97 | 0.183 | <0.001 | 0.448 |
| C20:0 | 0.05 [b] ± 0.04 | 0.08 [b] ± 0.03 | 0.44 [a] ± 0.04 | 0.07 [b] ± 0.03 | <0.001 | <0.001 | <0.001 |
| C14:1 *cis*-9 | 1.07 ± 0.24 | 1.49 ± 0.37 | 0.96 ± 0.15 | 1.11 ± 0.37 | 0.169 | 0.117 | 0.430 |
| C16:1 *cis*-9 | 3.76 ± 0.39 | 4.60 ± 1.43 | 3.84 ± 0.7 | 3.95 ± 0.71 | 0.587 | 0.361 | 0.484 |
| C16:1 *cis*-11 | 0.53 [bc] ± 0.003 | 0.68 [ab] ± 0.08 | 0.47 [c] ± 0.07 | 0.69 [a] ± 0.12 | 0.626 | 0.003 | 0.430 |
| C17:1 *cis*-9 | 0.71 ± 0.04 | 0.79 ± 0.04 | 0.76 ± 0.04 | 0.86 ± 0.04 | 0.183 | 0.095 | 0.839 |
| C18:1 *cis*-9 | 37.06 [a] ± 1.29 | 30.22 [b] ± 1.12 | 34.75 [a] ± 1.29 | 30.31 [b] ± 1.29 | 0.398 | 0.001 | 0.362 |
| C18:2 n-6 | 4.14 [a] ± 0.39 | 2.38 [b] ± 0.47 | 3.67 [ab] ± 0.47 | 4.50 [a] ± 0.47 | 0.131 | 0.356 | 0.036 |
| C18:2 *cis*-9, *trans*-11 | 0.29 [b] ± 0.07 | 0.25 [b] ± 0.05 | 0.49 [a] ± 0.07 | 0.37 [ab] ± 0.05 | 0.016 | 0.210 | 0.512 |
| C18:3 n-3 | 0.41 [c] ± 0.12 | 0.66 [bc] ± 0.09 | 0.8 [ab] ± 0.12 | 0.99 [a] ± 0.09 | 0.006 | 0.068 | 0.796 |
| C20:4 n-6 | 0.85 [b] ± 0.27 | 0.23 [b] ± 0.21 | 3.27 [a] ± 0.27 | 0.54 [b] ± 0.21 | <0.001 | <0.001 | 0.001 |
| C22:4 n-6 | 0.21 [a] ± 0.03 | 0.12 [b] ± 0.02 | 0.15 [ab] ± 0.03 | 0.17 [ab] ± 0.02 | 0.813 | 0.159 | 0.041 |
| C22:5 n-3 | 0.30 ± 0.05 | 0.22 ± 0.04 | 0.30 ± 0.05 | 0.29 ± 0.04 | 0.429 | 0.329 | 0.375 |

Values are expressed as mean ± standard error (SE). Different superscript letters indicate significant differences between means according to Fisher's LSD test ($p < 0.05$).

Overall, the IS chuck had higher polyunsaturated FA (PUFA) and n-6 PUFA family (n-6 PUFA) contents ($p < 0.05$), with 10.24 and 9.15 g FA/100 g total FA, respectively. This cut also had higher C18:2 *cis*-9, *trans*-11 and C20:4 n-6 contents (0.49 and 3.27 g FA/100 g total FA, respectively), as well as considerable C18:2 n-6 content (3.67 g FA/100 g total FA). Together, these three FA accounted for approximately 81% of the total n-6 PUFA. The higher PUFA content of IS chuck translated into more favourable values for ratios and indices linked to the unsaturated fraction, namely P/S, the peroxidability index (PI) and the

hypocholesterolemic/hypercholesterolemic (h/H) ratio (0.13, 23.87 and 0.32, respectively). Contrarily, the RFS chuck showed the highest n-3 PUFA content ($p < 0.05$; 1.28 g FA/100 g total FA), which is mainly due to its higher C18:3 n-3 content ($p < 0.05$; 0.99 g FA/100 g total FA), representing 77% of total n-3 PUFA. This profile suggests a more balanced PUFA composition from a nutritional standpoint.

The higher monounsaturated FA (MUFA) content in IS meat (42.92 g FA/100 g total FA) was primarily due to the greater contribution of C18:1 *cis*-9 (35.91 g of FA/100 g of total FA), in accordance with previously reported results [10]. IS also showed the highest C18:0 content (15.47 g FA/100 g of total FA). Although C18:0 is a SFA, it is considered to have a neutral effect on blood cholesterol, which may be regarded as an advantage in IS meat [10].

The n-6 PUFA family and n-3 PUFA family (n-6/n-3) ratio is relevant to human health because both n-3 and n-6 PUFA are precursors of eicosanoids with different health outcomes. Both families use the same enzymes for unsaturation and elongation; an excess of one can reduce the metabolism of the other [10,33,34]. This matters because n-3 FA give rise to eicosanoids with anti-atherogenic, antithrombotic, and anti-inflammatory properties [33,35–37], whereas n-6 FA tend to produce eicosanoids with more atherogenic, prothrombotic, and pro-inflammatory effects [34,36,38–40]. The IS meat showed a significantly higher ($p < 0.05$) n-6/n-3 ratio (8.56) than RFS meat (3.68), consistent with grain-fed and pasture-fed production systems, respectively [10,12]. The RFS value meets the recommended threshold ($\leq$4) [41], whereas the IS meat clearly exceeds this recommendation.

Meanwhile, sirloin RFS showed a higher atherogenicity index (AI) and thrombogenicity index (TI; 1.41 and 2.26, respectively). The higher AI and TI values likely reflect a greater contribution of SFA, especially C14:0 and C16:0 (5.45 and 32.2 g FA/100 g total FA, respectively), together with the higher overall fat content and the lower MUFA proportion in this meat. Taken together, these features suggest a higher atherogenic and thrombogenic potential [42]. However, the reasons for the higher fat content could not be identified, which is a limitation of this study.

RFS sirloin and chuck RFS showed the highest levels of iC17:0, although they differed significantly from each other ($p < 0.05$). These results align with the F-values, indicating that variation in iC17:0 is primarily explained by the production system (F = 74.6), while muscle type had no significant main effect ($p > 0.05$; F = 0.39). This finding is noteworthy because iC17:0 belongs to an emerging class of bioactive FA, known as methyl-branched-chain FA (BCFA), which have been associated with anti-carcinogenic and anti-inflammatory effects, as well as potential roles in metabolic regulation [43]. Accordingly, a higher iC17:0 proportion could be considered a potentially favourable nutritional attribute.

#### 3.3.2. Vitamin E

Because the MT $\times$ PS interaction was not significant ($p > 0.05$; Table 5) for total vitamin E and the quantified tocopherols (α-, β-, γ-, and δ-), the effects of MT and PS were interpreted as independent. Nevertheless, significant main effects of both factors were detected for total vitamin E and α-tocopherol ($p < 0.05$), indicating that each factor consistently influenced these variables. α-Tocopherol was the main tocochromanol detected, accounting for about 95–98% of total vitamin E. Its predominance is largely explained by the higher affinity of α-tocopherol transfer protein (α-TTP) for α-tocopherol relative to other tocochromanols [44].

**Table 5.** Tocopherol profile (expressed as mg/100 g of meat) in beef sirloin (*Longissimus lumborum*) and chuck (*Splenius capitis*) from different production systems (intensive system, IS; regenerative farming systems, RFS).

| | Sirloin | | Chuck | | *p* | | |
|---|---|---|---|---|---|---|---|
| | **IS** | **RFS** | **IS** | **RFS** | **MT** | **PS** | **MT × PS** |
| Total vitamin E | $0.20^{c} \pm 0.0797$ | $0.51^{b} \pm 0.0392$ | $0.28^{c} \pm 0.0795$ | $0.64^{a} \pm 0.1030$ | 0.026 | <0.001 | 0.541 |
| α-tocopherol | $0.19^{c} \pm 0.0684$ | $0.5^{b} \pm 0.0439$ | $0.27^{c} \pm 0.0789$ | $0.63^{a} \pm 0.0998$ | 0.020 | <0.001 | 0.572 |
| β-tocopherol | 0.002 ± 0.0007 | 0.005 ± 0.0004 | 0.001 ± 0.0005 | 0.001 ± 0.0005 | 0.133 | 0.258 | 0.432 |
| γ-tocopherol | 0.01 ± 0.0012 | 0.01 ± 0.0042 | 0.01 ± 0.0007 | 0.01 ± 0.0013 | 0.754 | 0.743 | 0.399 |
| δ-tocopherol | 0.001 ± 0.0009 | 0.002 ± 0.0005 | 0.002 ± 0.0005 | 0.001 ± 0.0007 | 0.727 | 0.433 | 0.095 |

Values are expressed as mean ± standard error (SE). Different superscript letters indicate significant differences between means according to Fisher's LSD test ($p < 0.05$).

Overall, total vitamin E and α-tocopherol were higher in RFS than in IS (0.58 and 0.56 mg/100 g of meat, respectively). In addition, when averaged across production systems, chuck showed higher total vitamin E and α-tocopherol than sirloin (0.51 and 0.49 mg/100 g of meat, respectively). Because vitamin E is lipophilic, variation in meat lipid content may partly explain differences in total vitamin E and α-tocopherol, with higher intramuscular fat potentially promoting greater vitamin E deposition [44]. Vitamin E remained higher in RFS across muscle types. This difference persisted even when fat content differences were modest between systems in chuck (RFS numerically higher), suggesting that α-tocopherol variation may not be explained solely by intramuscular fat.

In addition, FA composition can modulate vitamin E requirements, as more unsaturated lipids increase oxidative susceptibility and, consequently, antioxidant demand [45,46]. Nonetheless, this does not seem to account for our results, given that PUFA content was comparable in three of the four meat types.

Although α-tocopherol can reflect pasture intake, it is not uniquely specific to production systems because supplementation in intensive systems may also increase vitamin E. Therefore, in this study, vitamin E is interpreted as an observed compositional difference rather than a definitive marker of the production system. In the present study, only RFS meat, regardless of muscle type, exceeded the threshold (≥0.30 mg/100 g of tissue) proposed to prevent colour deterioration and lipid oxidation [47].

A key limitation is the lack of animal-level covariates (breed, age, sex, slaughter weight, carcass weight, finishing duration) inherent to a retail-based sampling design. These variables may confound observed differences and prevent causal inference regarding the production system. Accordingly, results are interpreted as a comparison of commercially available beef categories as labelled/marketed, and confirmation in controlled studies with full metadata is needed.

## 4. Conclusions

This exploratory study seems to indicate that beef produced under regenerative principles does not compromise nutritional quality in either sirloin or chuck. However, these results should be interpreted with caution due to limitations of the study, particularly the small sample size and the lack of detailed information on animal production conditions. Within these constraints, the results showed that meat from the regenerative farming system (RFS) showed higher n-3 PUFA levels and a more favourable n-6/n-3 ratio aligned with human health recommendations. Furthermore, RFS meat exhibited higher vitamin E concentrations, above the reference level reported in the literature for reduced lipid oxidation, which may be indicative of greater resistance to oxidative deterioration.

Overall, these findings support the potential of regenerative livestock systems to deliver beef with equal or improved nutritional quality. Nevertheless, given the commercial,

supply chain sourcing of samples and the limited sample size, the results should be considered preliminary and require confirmation in larger, controlled studies.

**Author Contributions:** Conceptualisation, I.D. and N.A.; methodology, I.C.A., L.C.R. and E.M.G.; software, I.C.A., L.C.R., C.A. and A.S.; validation, L.C.R., E.M.G. and C.A.; formal analysis, I.C.A., A.S. and H.G.; investigation, N.A. and I.D.; resources, N.A., J.R. and I.D.; data curation, A.S. and L.C.R.; writing—original draft preparation, I.C.A.; writing—I.C.A., L.C.R. and C.A.; visualisation, I.C.A. and L.C.R.; supervision, L.C.R., N.A. and I.D.; project administration, N.A., J.R., M.O. and I.D.; funding acquisition, N.A., M.O. and I.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by PRR—NextGenerationEU, Grant Projeto SPIN—Sustainable ProteIN (PRR-C05-i03-I-000192-LA9.5). The authors thank the Portuguese Foundation for Science and Technology (FCT) for the financial support to the Research Centre for Natural Resources, Environment and Society—CERNAS (UIDB/0681/2025).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

**Acknowledgments:** I. C. Antunes and J. Reis acknowledge Projeto SPIN—Sustainable ProteIN (PRR-C05-i03-I-000192-LA9.5) for supporting the research fellowship.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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