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Effect of Dietary Goji Berry (*Lycium barbarum*) Supplementation on Quality and Storage Stability of Rabbit Meat

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Featured Application

Dietary supplementation with goji berries can improve the nutritional quality and oxidative stability of rabbit meat rich in polyunsaturated fatty acids. The use of natural antioxidant-rich feed ingredients represents a practical strategy for extending meat shelf life during both refrigerated and frozen storage. These results support the application of goji berries as a functional feed additive in sustainable rabbit production systems.

Abstract

This study evaluated the effects of dietary supplementation with goji berries (*Lycium barbarum*) on the nutritional profile, oxidative stability, and shelf life of rabbit meat. Thirty-two rabbits were assigned to two dietary treatments: a control diet (CN) and the same diet supplemented with 3% dried goji berries (GJ). Proximate composition and fatty acid profile of the *Longissimus thoracis et lumborum* muscle were determined at dissection, whereas physical, microbiological, and biochemical parameters were evaluated during refrigerated storage (4 °C; 1, 4, and 10 days) and frozen storage (−20 °C; 60 and 120 days). Dietary supplementation significantly modified the lipid profile of the meat, reducing saturated fatty acids and increasing long-chain n-3 and n-6 polyunsaturated fatty acids. During refrigerated storage, lipid peroxidation increased in both groups; however, meat from the GJ group showed significantly lower TBARS values after 10 days (0.22 vs. 0.33 mg MDA/kg; $p < 0.001$), indicating improved oxidative stability. Lower accumulation of total volatile basic nitrogen (TVB-N), reduced formation of biogenic amines, and slower growth of spoilage-related microbial populations, particularly *Pseudomonas* spp., were also observed in GJ samples. Overall, the GJ diet improved fatty acid composition and delayed degradative processes during storage, suggesting its potential as a functional feed ingredient to enhance rabbit meat quality and shelf life.

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1. Introduction

In modern meat production systems, preserving product quality and extending shelf life have become essential priorities, driven by evolving consumer habits and the logistical demands of large-scale retail distribution. These challenges are particularly relevant for meats naturally rich in polyunsaturated fatty acids (PUFAs), which are inherently susceptible to oxidative degradation [1]. Fluctuations during storage and processing can accelerate oxidative processes, leading to the deterioration of both sensory and nutritional properties, as well as indirectly compromising the microbiological stability of the meat. Oxidative damage not only alters meat colour and lipid structure but also promotes the accumulation of harmful secondary products such as malondialdehyde and carbonyl compounds [2]. Furthermore, these processes facilitate the formation of biogenic amines, including cadaverine and histamine [3,4], while reducing the preservation of physiological polyamines (PAs), such as spermine and spermidine [5], as well as endogenous antioxidant compounds such as thiol groups [6]. In this context, livestock nutritional strategies capable of reinforcing endogenous antioxidant defences represent a proactive and sustainable approach to improve both the technological stability and the safety of meat products [7].

Over the past decades, animal nutrition has progressively evolved from a production-oriented model toward a broader One Health perspective, in which animal welfare, food quality, environmental sustainability, and public health are regarded as interrelated priorities [8]. Within this integrated framework, plant-based feed additives rich in bioactive compounds, including polyphenols, flavonoids, carotenoids, and antioxidant vitamins, have demonstrated promising potential in modulating lipid metabolism, attenuating inflammatory responses, and strengthening antioxidant capacity across various livestock species [9].

Among the plant-derived functional feed ingredients of increasing interest, goji berries, obtained from *Lycium barbarum*, a plant belonging to the *Solanaceae* family and traditionally used in Chinese medicine, have attracted considerable attention [10–12]. Goji berries have been reported to contribute to redox homeostasis and to exert beneficial metabolic effects, particularly in relation to lipid metabolism [13]. Their rich phytochemical profile includes *Lycium barbarum* polysaccharides, carotenoids, particularly zeaxanthin, flavonoids, phenolic acids, betaine, vitamins, and essential minerals [12–15]. In this regard, *Lycium barbarum* polysaccharides have been shown to potentially activate key metabolic regulators, including AMPK (AMP-activated protein kinase) and SIRT1 (sirtuin 1, a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase) [16,17]. However, the improvements in liver and muscle lipid profiles associated with goji supplementation do not appear to result from a direct modulation of fatty acid metabolic pathways. Instead, they may reflect the establishment of a favourable cellular redox environment characterized by reduced oxidative stress, which could support hepatic enzymatic activity and promote the retention of long-chain PUFAs in tissues [18].

Consistently, goji supplementation has been reported to support redox homeostasis by contributing to the maintenance of hepatic thiol pools and glutathione levels, thereby reinforcing antioxidant defence mechanisms [14,19]. However, contrasting evidence has also been reported, as Jianke et al. [20] observed reduced hepatic glutathione levels in mice receiving goji berry supplementation, highlighting the influence of experimental conditions on the biological response.

While most studies on goji supplementation have been conducted in poultry and swine [21,22], recent investigations by our research group have begun to explore its dietary application in rabbits [23–25]. Rabbit meat, classified as a white meat of high nutritional value, is characterized by a low total fat content and a favourable fatty acid profile, with relatively high levels of polyunsaturated fatty acids. While this compositional feature enhances its nutritional quality, it also increases susceptibility to oxidative damage, which may indirectly contribute to microbial spoilage during refrigerated storage [26,27]. Preliminary evidence suggests that dietary goji supplementation may improve the oxidative stability of rabbit meat under chilled conditions [23]. However, direct evidence regarding its effects on the fatty acid profile of rabbit meat remains limited, and information on its potential role in limiting the formation of oxidative degradation products, such as protein carbonyls and biogenic amines, is still scarce in this species.

Given these premises, the inclusion of goji berries in rabbit diets was hypothesized to be a potentially valuable nutritional strategy, owing to their bioactive and antioxidant properties. The present study seeks to provide new insights into the effects of dietary goji supplementation on rabbit meat quality by investigating potential modifications in the fatty acid profile, with particular attention to PUFA content. In addition, the study aims to evaluate the impact of diet supplemented with goji berries on the oxidative and microbiological stability of rabbit meat during refrigerated and frozen storage, including the formation of biogenic amines.

2. Materials and Methods

2.1. Animals and Diets

The study was carried out at the experimental rabbit farm of the Faculty of Veterinary Medicine, Agricultural University of Tirana (Albania). All animals were managed in accordance with Legislative Decree No. 146/2001, implementing Directive 98/58/EC on the protection of animals kept for farming purposes. The experiment was carried out following authorization from the Albanian Ministry of Agriculture and Rural Development and approval by the National Authority of Veterinary and Plant Protection (protocol no. 824/2021).

The trial consisted of two experimental cycles conducted in two different periods. In each cycle, 16 male New Zealand White rabbits were used, for a total of 32 animals. After weaning, at 35 days of age (average body weight: 832.26 ± 64.13 g), rabbits were individually housed in conventional wire cages (75 × 38 × 25 cm, length × width × height) under controlled environmental conditions. Rabbits were randomly allocated to two experimental groups (n = 16 animals per group), in a completely randomized design with diet as the single experimental factor, each receiving a different pelleted diet: CN (control group), fed a commercial diet, and GJ, fed the same diet supplemented with 3% dried goji berries. The composition of the diets is reported in Table 1. The experimental period lasted from 42 to 85 days of age (starting one week after weaning), during which animals received a daily pellet feed allowance that was progressively increased by 12% per week, from 100 g/day to 197.5 g/day. Fresh water was available ad libitum throughout the entire rearing period.

The fatty acid profiles of the two diets and goji berries are reported in Table S1. All diets were formulated to be isoenergetic and isonitrogenous, meeting the nutritional requirements for growing–fattening rabbits, according to current recommendations [28]. The inclusion level of 3% goji berries was selected based on previous experimental evidence indicating this level as the most effective for improving oxidative stability parameters, whereas a lower inclusion level (1%) did not produce significant effects compared to the control group [24].

Table 1. Formulation and chemical composition of the two experimental diets.

Ingredients (%)	CN	GJ
Dried goji berries	-	3.00
Corn grain	20.00	19.00
Barley	18.00	17.50
Soybean meal	12.00	11.00
Wheat bran	15.00	14.50
Alfalfa hay	30.00	30.00
Sunflower oil	1.00	1.00
Cane molasses	2.00	2.00
Calcium carbonate	0.90	0.90
[1] Premix vitamin	0.50	0.50
Lysine	0.23	0.23
Met + Cys	0.15	0.15
L Threonine	0.07	0.07
Sodium chloride	0.15	0.15
Chemical compounds (g/100 g of pellet)		
Dry matter	89.58	89.28
Ash	7.12	7.15
Crude protein	17.01	16.98
Ether extract (EE)	3.56	3.58
Crude fibre	13.66	13.21
N-free extract (NFE)	48.23	48.36
NDF	32.01	32.18
ADF	15.97	16.14
ADL	3.11	3.13
Digestible energy (Kcal/kg)	2234.4	2235.2

Met + Cys: methionine + cysteine; [1] Premix composition: Per kg diet: vitamin A 11,000 IU; vitamin D3 2000 IU; vitamin B1 2.5 mg; vitamin B2 4 mg; vitamin B6 1.25 mg; vitamin B12 0.01 mg; alpha-tocopherol acetate 50 mg; biotin 0.06 mg; vitamin K 2.5 mg; niacin 15 mg; folic acid 0.30 mg; D-pantothenic acid 10 mg; choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg. NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Acid Detergent Lignin. CN = control diet; GJ = CN + 3% dried goji berries.

The goji berries (*Lycium barbarum* L.) used in this study were supplied in dried form by a local Italian producer (Impresa Agricola Gianluca Bazzica, Foligno, Italy). According to the producer, the berries were harvested during the typical ripening season (September–October) under Mediterranean conditions and subjected to post-harvest dehydration by controlled hot-air drying to reduce moisture content and ensure shelf stability. Prior to feed preparation, the dried berries were mechanically processed into small fragments, as previously described by Castrica et al. [23], and subsequently incorporated into the diet mixture. The complete feed was then pelleted using a farm-scale pelletizer and dried before administration. Detailed information on the main bioactive compounds of the goji berries used in this study was provided by the producer and is reported in Table S2.

After a 12 h feed withdrawal, rabbits at about 85 days of age were weighed and slaughtered in an authorized commercial abattoir. Animals were stunned by electronarcosis, in accordance with standard animal welfare practices and in compliance with Council Regulation (EC) No 1099/2009. Immediately following stunning, the animals were suspended by the hind legs on the processing line and rapidly exsanguinated by severing the jugular veins and carotid arteries. The skin, distal parts of the limbs, gastrointestinal tract, visceral fat, and urogenital tract were removed to obtain the commercial carcass, while the head, liver, kidneys, heart, and lungs were retained. Carcasses were then chilled at 4 °C immediately after slaughter.

2.2. Chemical Analyses of Pelleted Feed

Approximately 500 g of each pelleted feed was finely ground using a laboratory mill (Cemotec 1090 sample mill, FOSS, Hilleroed, Denmark) to obtain a homogeneous sample with uniform particle size and composition. Feed samples were analyzed for proximate composition, including dry matter (DM; AOAC 930.15), ash (AOAC 942.05), crude protein (CP; AOAC 990.03), ether extract (EE; AOAC 945.16), and crude fibre (CF; AOAC 978.10), according to the Official Methods of Analysis [29].

Ether extract content was determined using a Soxhlet extraction system (Tecator Soxtec System HT 1043 extraction unit, Gemini, Apeldoorn, The Netherlands). Crude protein (N × 6.25) was quantified using the Kjeldahl method with a Tecator Digestion System and a Kjeltec Auto 1030 Analyser (Tecator, Apeldoorn, The Netherlands). Fibre fractions were analyzed using a FIWE Fibre Extractor (Velp Scientifica, New York, NY, USA). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to the method described by Van Soest et al. [30]. All chemical constituents were expressed as g/100 g of pellet. Nitrogen-free extract (NFE) was calculated by difference, subtracting crude protein, ether extract, crude fibre, and ash from dry matter. Based on the analyzed chemical composition, the digestible energy (kcal/kg) of the experimental diets was estimated using the predictive equation recommended by European guidelines for rabbit nutrition [27].

$$\text{Digestible Energy (Kcal/Kg)} = 1801 + 7.10 \times \text{CP} + 12.01 \times \text{EE} + 5.59 \times \text{NFE}$$

where CP = crude protein, EE = ether extract, and NFE = nitrogen-free extract.

Fatty acids were also analyzed by GC-FID following the procedures described for meat samples.

2.3. Carcass Composition and Meat Sampling

Sixteen carcasses per experimental diet were transported to the Italian laboratory under controlled refrigerated conditions. Upon arrival, carcasses were weighed 24 h post-mortem to obtain the chilled carcass weight (CCW) and subsequently dissected according to the recommendations of the World Rabbit Science Association (WRSA) [31]. In addition, the individual weights of the liver, kidneys, pelvic, and scapular fat were determined. A portion of the liver was immediately stored at −80 °C and later used for glutathione analysis. Carcass composition was further evaluated by determining the relative proportions of the main anatomical regions: the fore part (FLP), thoracic region (TTP), and hind part (HPP). In addition, the right hind leg was separated and dissected into its main tissue components (bone, muscle, and the fraction consisting of intermuscular fat and connective tissues), which were individually weighed, allowing the calculation of the relative percentages of tissues. Finally, the meat-to-bone ratio of the hind leg was calculated.

Following carcass dissection, both *Longissimus thoracis et lumborum* (LTL) muscles were excised and processed according to the sampling design illustrated in Figure 1. The distal portions of the two LTL were separated, minced, and stored at −80 °C for proximate composition and fatty acid profile analyses. The remaining proximal portions were divided into five samples of comparable size. One sample was analyzed at 1-day post-dissection (1 T). Two samples were stored under refrigerated conditions (4 °C) and analyzed after 4 and 10 days (4 T and 10 T), while two additional samples were frozen at −20 °C and analyzed after 60 and 120 days (60 T and 120 T).

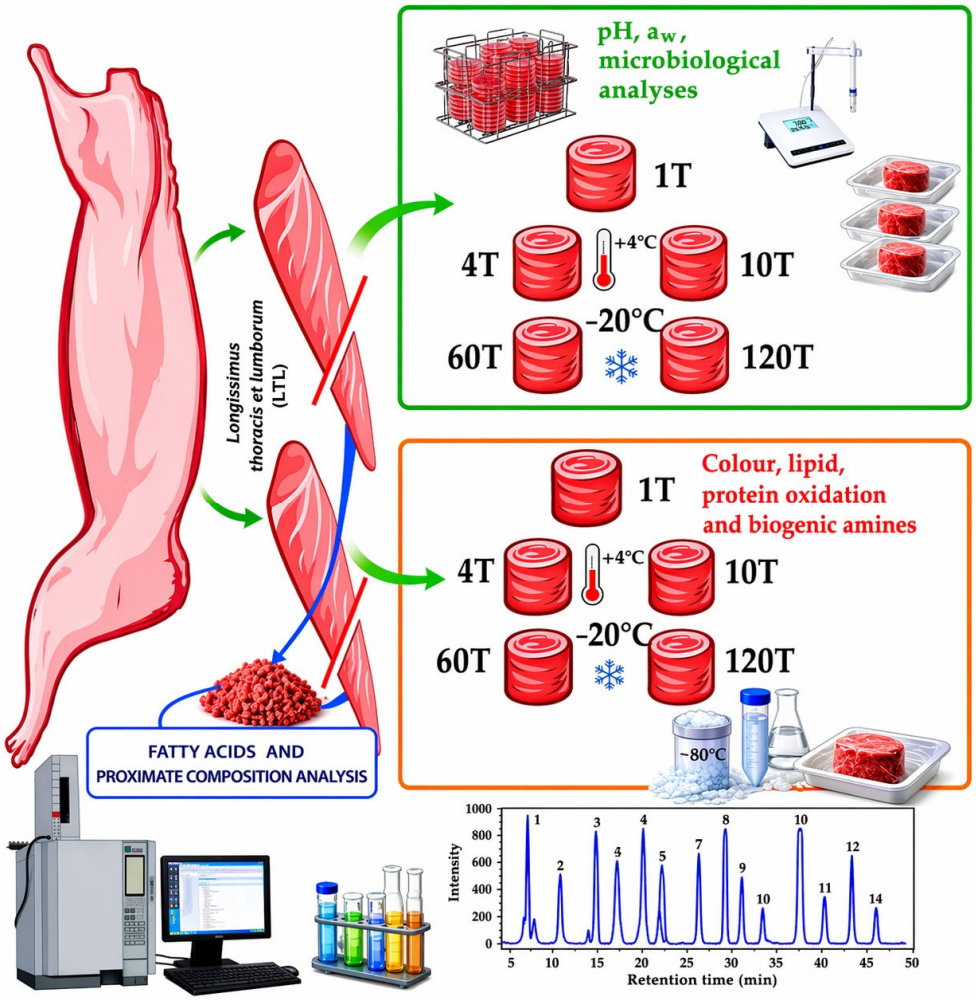

Figure 1. Subdivision of the *Longissimus thoracis et lumborum* (LTL) muscle and sampling design. The distal portion of each LTL was minced and used for proximate composition and fatty acid analyses. The remaining portion was divided into five parts of comparable size. One LTL was used for microbiological analyses, pH, and water activity (a_w), whereas the other LTL was used for lipid and protein oxidation analyses and for biogenic amine analysis. The samples were analyzed at 1-day post-dissection (1 T), after refrigerated storage (4 and 10 days at 4 °C; 4 T, 10 T), and after frozen storage (60 and 120 days at −20 °C; 60 T, 120 T). The figure provides a schematic overview of the we have not sampling design and analytical workflow.

At each sampling time, pH, water activity (a_w), microbiological parameters, drip loss, thaw loss, and colour measurements were determined. After these determinations, samples were frozen at −80 °C for subsequent chemical analyses, including lipid oxidation by thiobarbituric acid reactive substances (TBARSs), protein oxidation markers (thiol groups and carbonyl content), total volatile basic nitrogen (TVB-N), and biogenic amines. Due to the number of samples, analyses were carried out on samples stored at −80 °C for up to two months, a condition that ensured oxidative stability and preserved sample integrity. The muscle subdivision and the sampling procedure are illustrated in Figure 1.

2.4. Chemical Analyses of LTL Muscle at Dissection

All reagents were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All reagents and solvents used were of analytical grade.

2.4.1. Proximate Composition

The proximate composition of the meat samples was determined following standard AOAC procedures [29], including dry matter (DM; AOAC 950.46), crude fat (CF; AOAC 954.02), ash (AOAC 942.05), and crude protein (CP; AOAC 990.03). The same analytical instruments (Soxhlet and Kjeldahl) described for proximate analysis of pellet were used for crude fat and crude protein determination in meat.

2.4.2. Glutathione Analysis

Glutathione (GSH) content in rabbit liver was determined according to Nuhu et al. [32] for extraction and by Niero et al. [33] for derivatization and chromatographic conditions. Briefly, liver samples were homogenized in 6% perchloric acid and centrifuged (NEYA 10R, Giorgio Bormac s.r.l., Modena, Italy), and the supernatant was derivatized with ammonium 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F). The derivatized samples were analyzed by HPLC (Alliance 2695, Waters Corporation, Framingham, MA, USA) with fluorescence detection (λ_ex = 386 nm; λ_em = 516 nm). Results were expressed as μg GSH/g tissue.

2.4.3. Fatty Acid Profile

Total lipids were extracted from approximately 10 g of minced LTL muscle using a chloroform: methanol mixture (2:1, v/v). After extraction, lipids were transesterified with a 2 M methanolic KOH solution to obtain fatty acid methyl esters (FAMEs). FAMEs were analyzed by gas chromatography (GC 6890N, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm internal diameter, 0.20 μm film thickness; Agilent Technologies). Detailed extraction and chromatographic conditions were described by Failla et al. [34]. Nonadecanoic acid (C19:0) was added as an internal standard before lipid extraction. Individual FAMEs were identified by comparing their retention times with those of certified standard mixtures (Supelco 37 FAME Mix and docosapentaenoic acid; Sigma-Aldrich Merck, Darmstadt, Germany) and expressed as percentages of total identified FAMEs and as mg/g of meat.

Based on the fatty acid profile, the n-6/n-3 ratio and the atherogenic (AI) and thrombogenic indices (TI) were calculated according to the equations proposed by Ulbricht and Southgate [35]. The peroxidation index (PI) was calculated as described by Arakawa and Sagai [36]. Fatty acids were finally grouped into saturated (∑ SFA), monounsaturated (∑ MUFA), and polyunsaturated fatty acids (∑ PUFA), including PUFA n-6 and PUFA n-3, as well as their respective long-chain fractions (∑ LC-PUFA n-6 and ∑ LC-PUFA n-3). Apparent metabolic activities related to fatty acid desaturation were estimated using the product-to-precursor ratios. In particular, Δ9 desaturase activity at the n-7 position and along the n-9 pathway was calculated. Apparent Δ6 desaturase activity was estimated for the n-6 and n-3 series, whereas apparent Δ5 desaturase activity for the same series was calculated following the approach described by Vessby et al. [37]. Elongase activity indices were calculated according to the ratios proposed by Mota-Martorell et al. [38], highlighting the elongation of fatty acids from 16:0 to 18:0 (ELOVL6), from 20:4n-6 to 22:4n-6 (ELOVL2/5, n-6), and from 20:5n-3 to 22:5n-3 (ELOVL2/5, n-3).

2.5. Analyses to Estimate Shelf Life of Meat During Storage

2.5.1. Physical Analyses

pH was measured using a portable pH metre (model 110; Eutech Instruments, Singapore) equipped with a Double Pore D electrode (Hamilton, Bonaduz, Switzerland). Water activity (a_w) was measured using an AquaLab Series 3 instrument (Decagon Devices, Pullman, WA, USA). Drip loss and thaw loss were calculated as the difference between the

initial sample weight recorded at sampling and the weight measured after the predetermined storage period.

Meat colour was evaluated on the LTL muscle after 30 min of air exposure to allow colour blooming, using the CIELAB colour space (International Commission on Illumination, CIE), by recording lightness (L*), redness (a*), and yellowness (b*). Measurements were performed using a Konica Minolta CM-3600D Spectro colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), equipped with a D65 illuminant (6504 K, daylight). Hue angle (H), chroma (C), and total colour difference (ΔE) were calculated from L*, a*, and b* coordinates as described in detail by Ripoll et al. [39]. Colour data were expressed as the average of four readings taken at different points on each sample.

2.5.2. Microbiological Analyses

Raw LTL samples were examined for the presence of naturally occurring microbial populations. Samples were analyzed at the following storage times: 1, 4, and 10 days at 4 °C, as well as 60 and 120 days at −20 °C. Briefly, 5 g of each raw LTL sample were diluted in 45 g of sterile Ringer's solution (Liofilchem, Teramo, Italy) and homogenized using a stomacher (Seward, London, UK) for 2 min at room temperature. Homogenates were serially ten-fold diluted, and 1.0 or 0.1 mL aliquots of appropriate dilutions were either pour-plated or spread-plated onto the following culture media for microbial enumeration: total viable counts (TVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, *Lactobacillus* spp., *Enterobacteriaceae*, and total coliforms, according to Castrica et al. [23] and Valerio et al. [40].

Results were expressed as $\log_{10}$ colony-forming units per gram ($\log_{10}$ CFU/g). The detection limit of bacterial enumeration was ≥10 CFU/g.

2.5.3. Chemical Analyses

Thiobarbituric Acid Reactive Substances (TBARSs)

Lipid oxidation was evaluated by TBARS assay, using malondialdehyde (MDA) as the reference compound. Briefly, an aliquot of the supernatant obtained after sample dilution and protein precipitation with trichloroacetic acid (TCA) was incubated with thiobarbituric acid (TBA) solution (0.28%). The resulting reaction mixture was injected into an HPLC system (Alliance 2695, Waters Corporation, Framingham, MA, USA) with fluorescence detection (λ_ex = 515 nm and λ_em = 543 nm). The MDA–TBA adduct was identified by comparison with an external MDA standard, and TBARS values were expressed as mg MDA/kg of meat, as described by Valerio et al. [40].

Thiol and Carbonyl Content

Protein oxidation was evaluated by determining thiol (SH) and carbonyl (CO) group contents in meat samples, following the method described by Valerio et al. [40]. Thiol content was determined by homogenizing 1 g of meat in 0.15 M potassium chloride. Aliquots were reacted with 2,2′-dithiodipyridine (DTNP) in urea buffer, using a corresponding blank without DTNP. After incubation in the dark for 1 h, absorbance was measured at 386 nm. Results were expressed as nmol SH/mg protein. Carbonyl groups were quantified by homogenizing 1 g of meat in phosphate buffer. Aliquots were treated with 2 N HCl (blank) or 0.2% dinitrophenylhydrazine (DNPH) in 2 N HCl. After incubation, samples were washed with ethanol:ethyl acetate (1:1, *v*/*v*) and the pellets dissolved in 6 M guanidine HCl. Absorbance was measured at 280 nm (blank) and 370 nm (DNPH-treated samples), and carbonyl content was expressed as nmol DNPH/mg protein.

Total Volatile Basic Nitrogen (TVB-N) by Conway's Microdiffusion Method

TVB-N was determined according to the method described by Siang and Kim [41] using Conway's microdiffusion method, with minor modifications. Briefly, 10 g of sample

was homogenized with trichloroacetic acid (TCA) and then centrifuged and filtered. The solution of filtrate and saturated potassium carbonate was transferred into the outer ring of a Conway's unit, and 1% of boric acid was added to the inner ring. After incubation, the inner ring solution was titrated with 0.01 N_{HCl}. The TVB-N was calculated according to the following equation:

$$\text{mg of TVB-N}/100\text{ g meat} = (Vs - Vb) \times (N_{HCl} \times An) \times [(Ws \times (M/100)) + V_E] \times 100/Ws$$

where Vs = Titration volume of 0.02 N_{HCl} for sample extract (mL); Vb = Titration volume of 0.02 N_{HCl} for blank (mL); N_{HCl} = Normality of HCl (=0.02 N × f, factor of HCl); An = Atomic weight of nitrogen (14.00); Ws = Weight of muscle sample (g); M = Percentage moisture of muscle sample; V_E = Volume of TCA used in extraction.

Biogenic Amines (BAs)

The content of biogenic amines (cadaverine, histamine, and spermine) were selected based on their complementary biological and technological relevance. Histamine represents the most toxicologically significant BA and is widely used as an indicator of food safety. Cadaverine is associated with microbial spoilage and protein degradation, whereas, spermine is a physiological polyamine involved in cellular metabolism and is generally more abundant in meat than spermidine [3–5].

BAs in LTL samples were determined at each sampling time by HPLC. Briefly, 1 g of meat was extracted with 0.2 M trichloroacetic acid (TCA) and centrifuged, and the supernatant analyzed in duplicate following OPA/N-acetylcysteine (OPA/NAC) derivatization, according to Learey et al. [42]. Chromatographic separation was performed by HPLC system (Agilent 1260 Infinity Binary LC) with fluorescence detection (λ_ex = 330 nm; λ_em = 440 nm). Quantification was carried out using external calibration curves, and results were expressed as mg/kg of meat. Limits of detection (LOD) and quantification (LOQ) were calculated according to Jajić et al. [43].

2.6. Statistical Analysis

All statistical analyses of chemical data were performed using SAS Systems statistical software v.9.4 (SAS Institute Inc., Cary, NC, USA). Data collected at dissection were analyzed by one-way analysis of variance with diet (CN vs. GJ) as fixed effect, implemented with PROC GLM, and significant differences were assessed using Tukey–Kramer adjustment for multiple comparisons.

Shelf life parameters were analyzed using linear mixed models (PROC MIXED) including diet (CN vs. GJ), storage time with interaction as fixed effects, and animal as a random effect to account for repeated measurements. To avoid confounding between storage conditions, refrigerated samples (1, 4, and 10 days at 4 °C) and frozen samples (1 day at 4 °C, 60 and 120 days at −20 °C) were analyzed separately. Statistical analyses of microbiological data were conducted using Statistica v.13 (Dell Inc., Round Rock, TX, USA, 2015). Microbiological results are reported as $\log_{10}$ CFU/g. Microbial population data were analyzed by ANOVA using a two way with interaction model. Values below the detection limit (<DL) were assigned a value of 0.5 (half the DL) for analysis. Least squares means were compared using Tukey–Kramer adjustment for multiple comparisons.

Principal Component Analysis (PCA) was performed on standardized variables to explore multivariate relationships among physicochemical and oxidative parameters associated with shelf life. Data were log-transformed prior to analysis to avoid scale-driven bias and ensure equal weighting of variables. Because of missing values in several microbiological parameters, only the total viable count was included in the PCA.

3. Results and Discussion

3.1. Effect of Diet on Carcass Composition

The macronutrient composition of the GJ diet was comparable to that of the CN diet. Accordingly, the inclusion of goji berries in the GJ diet did not result in significant differences in dietary protein and energy content (Table 1). This nutritional similarity likely contributed to the absence of significant differences in live weight at slaughter, which averaged 2209.53 ± 146.41 g in the CN group and 2321.14 ± 163.03 g in the GJ group. Despite the similar slaughter weights, dietary goji supplementation significantly influenced several carcass characteristics (Table 2). Rabbits fed the GJ diet showed a higher carcass weight compared to the CN group (1265.90 ± 56.14 vs. 1218.28 ± 64.81 g; $p < 0.05$). This difference may be associated with differences in non-carcass components removed prior to carcass weighing, particularly gastrointestinal contents and visceral fat deposits. However, as these components were not directly measured, this interpretation remains speculative.

Table 2. Carcass characteristics and tissue composition of rabbits fed two experimental diets.

	CN	GJ	*Significance*
CCW g	1218.28 ± 64.81	1265.90 ± 56.14	*
FLP%	28.17 ± 0.89	27.31 ± 0.76	**
TTP%	34.52 ± 0.68	35.05 ± 0.78	*
HPP%	37.30 ± 0.87	37.63 ± 1.04	ns
Meat%	75.73 ± 0.36	76.25 ± 0.41	**
Bone%	21.54 ± 0.46	21.48 ± 0.32	ns
Fat%	2.73 ± 0.42	2.27 ± 0.31	**
Meat/bone	3.51 ± 0.09	3.55 ± 0.07	ns

CCW = chilled carcass weight; FLP = fore part; TTP = thoracic region; HPP = hind part; CN = control diet; GJ = CN + 3% dried goji berries; values are expressed as mean ± standard deviation; statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, ns = not significant.

The distribution of carcass cuts was also affected by the diet, with a lower proportion of the fore part (FLP%) in GJ rabbits ($p < 0.01$) and a slightly higher thoracic part proportion (TTP%) ($p < 0.05$), whereas the hind part proportion (HPP%) remained unchanged ($p > 0.05$). Goji supplementation was associated with a higher estimated meat yield and a lower carcass fat percentage, whereas bone percentage and meat-to-bone ratio were unaffected. These findings are consistent with previous evidence suggesting an effect of goji supplementation on body fat deposition [16,17].

Dietary treatment did not affect the weight of internal organs, such as liver and kidney weights, which are included in the CCW, according to standard rabbit carcass evaluation procedures [31], suggesting comparable organ development between both groups. The literature on the effects of dietary goji supplementation on rabbit carcass characteristics remains limited. Most studies conducted in rabbits have primarily focused on growth performance, reproductive traits, and meat oxidative stability, rather than on detailed carcass evaluation [23,24]. The results of the present study highlight a positive qualitative effect of goji berry supplementation on carcass composition, characterized by a higher meat percentage and reduced fat deposition. These findings are consistent with previous studies reporting reduced intramuscular fat accumulation and improved muscle traits following goji supplementation. Such effects have been attributed to the bioactive components of goji, particularly *Lycium barbarum* polysaccharides (LBPs) such as arabinogalactans, which have been reported to modulate lipid metabolism and reduce fat deposition, potentially explaining the shift toward leaner carcasses observed in the GJ group [16,17].

3.2. Chemical Composition of the Longissimus Thoracis et Lumborum Muscle at Dissection

3.2.1. Effect of Diet on Proximate Composition and GSH in Liver

The proximate composition of the rabbit LTL muscle was only marginally affected by dietary treatment (Table 3). Moisture, protein, and ash contents did not differ between groups ($p > 0.05$). In contrast, the LTL of the GJ group exhibited a lower crude fat content compared with the CN group (1.07 ± 0.13% vs. 1.22 ± 0.14%; $p < 0.05$). This finding is consistent with the reduced adipose tissue deposition in the GJ group and aligns with the results reported by Zhan et al. [44] in sheep.

Table 3. Proximate composition of *Longissimus thoracis et lumborum* muscle and GSH in the liver of rabbits fed two experimental diets.

	CN	GJ	*Significance*
Moisture %	76.50 ± 0.33	76.70 ± 0.49	ns
Crude protein %	21.23 ± 0.34	21.18 ± 0.51	ns
Crude fat %	1.22 ± 0.14	1.07 ± 0.13	*
Ash %	1.06 ± 0.09	1.05 ± 0.08	ns
GSH (μg/g of liver)	153.43 ± 28.51	193.82 ± 55.74	ns ¥

GSH = Reduced glutathione; CN = control diet; GJ = CN + 3% dried goji berries; values are expressed as mean ± standard deviation; statistical significance is indicated as follows: * $p < 0.05$, ¥ indicates a tendency ($0.05 \leq p < 0.10$), and ns = not significant.

In addition, goji supplementation is known to exert antioxidant effects, which may contribute to the preservation of hepatic GSH. As a major intracellular antioxidant, GSH plays a central role in maintaining redox homeostasis, supporting detoxification pathways and overall liver function [45,46]. Although the difference did not reach statistical significance, rabbits in the GJ group exhibited higher hepatic GSH concentrations compared with the CN group (193.8 vs. 153.4 μg/g liver; $p = 0.082$), suggesting a trend toward improved redox status.

3.2.2. Effect of Diet on Fatty Acid Profile

Although the experimental diets were isoenergetic and comparable in macronutrient composition, the inclusion of goji berries significantly influenced the fatty acid profile of the LTL muscle (Table 4). These changes may be attributable to the bioactive compounds provided by goji berries. Specifically, the GJ group showed a lower content of total SFA compared with the CN group (38.09% vs. 40.44% for GJ and CN, respectively; $p = 0.002$), mainly due to a reduced proportion of 16:0 (−1.35%), whereas 18:0 (stearic acid) remained unchanged ($p > 0.05$). Since 16:0 (palmitic acid) represents the primary end-product of de novo lipogenesis, its reduction suggests a moderate attenuation of endogenous fatty acid synthesis in the goji-supplemented group, in line with previous evidence on the metabolic effects of goji polysaccharides [16,17].

Total MUFAs were not influenced by dietary treatment ($p > 0.05$), including oleic acid (18:1 n-9) and trans-MUFAs such as trans-vaccenic acid (Table S3). Likewise, no differences were observed in branched-chain fatty acids or conjugated linoleic acid (CLA) isomers, lipid fractions typically associated with microbial metabolism. These findings differ from those reported by Zhan et al. [44] in small ruminants, where goji supplementation modified several MUFAs and microbially derived fatty acids. However, these discrepancies are likely attributable to species-specific differences in digestive physiology. Although Cremonesi et al. [25] demonstrated that goji supplementation can modulate the caecal microbiota in rabbits, such modulation did not translate into measurable changes in the accumulation of these microbial-derived fatty acids in the meat.

Table 4. Fatty acid profile expressed as percentage of total FAMEs of *Longissimus thoracis et lumborum* muscle of rabbits fed two experimental diets.

%	CN	GJ	*Significance*
∑ SFA	40.44 ± 1.48	38.09 ± 1.62	**
16:0	29.77 ± 1.05	28.63 ± 1.29	*
18:0	6.93 ± 0.39	6.82 ± 0.47	ns
∑ Branch + odd	1.69 ± 0.15	1.62 ± 0.20	ns
∑ MUFA	30.85 ± 1.14	30.31 ± 0.92	ns
18:1 n-9	22.72 ± 0.90	22.53 ± 0.76	ns
∑ MUFA trans	0.43 ± 0.07	0.39 ± 0.09	ns
∑ PUFA n-6	22.67 ± 0.55	24.82 ± 1.61	***
18:2 n-6	18.69 ± 0.53	19.12 ± 0.74	ns ¥
LC-PUFA n-6	3.98 ± 0.61	5.60 ± 1.08	***
∑ PUFA n-3	2.42 ± 0.16	3.21 ± 0.18	***
18:3 n-3	1.32 ± 0.14	1.40 ± 0.13	ns
LC-PUFA n-3	1.10 ± 0.18	1.81 ± 0.21	***
∑ PUFA	25.09 ± 0.56	28.03 ± 1.68	***
∑ CLA	0.09 ± 0.03	0.08 ± 0.03	ns
n-6/n-3	9.43 ± 0.71	7.74 ± 0.51	***

FAME = fatty acid methyl ester; ∑ SFA = saturated fatty acid—12:0, 14:0, 16:0, 18:0, 20:0, 21:0, 22:0, 24:0; ∑ Branch + odd = 15:0iso, 15:0anteiso, 16:0iso, 17:0iso, 17:0anteiso, 18:0iso, 17:0, 15:0; ∑ MUFA = monounsaturated fatty acid—14:1, 15:1, 16:1 n-9, 16:1 n-7, 17:1 n-7, 18:1 n-9, 18:1 n-7, 20:1 n-9, 20:1 n-7, 22:1 n-11, 24:1 n-15; ∑ MUFA trans = 18:1trans-9, 18:1trans-11; ∑ PUFA n-6 = Polyunsaturated fatty acids omega-6—18:2 n-6, 18:2 n-6 trans isomers, 18:3 n-6, 20:2 n-6, 20:3 n-6, 20:4 n-6, 22:2 n-6, 22:4 n-6; LC-PUFA n-6 = Long-chain fatty acids omega-6—20:2 n-6, 20:3 n-6, 20:4 n-6, 22:2 n-6, 22:4 n-6; ∑ PUFA n-3 = polyunsaturated fatty acids omega-3—18:3 n-3, 20:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3; LC-PUFA n-3 = Long-chain fatty acids omega 3—20:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3; ∑ CLA = conjugated linoleic acids; 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 n-9 = oleic acid; 18:2 n-6 = linoleic acid; 18:3 n-3 = linolenic acid; n-6/n-3 = PUFA omega-6/PUFA omega-3. CN = control diet; GJ = CN + 3% dried goji berries; values expressed as mean ± standard deviation; statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ¥ indicates a tendency ($0.05 \leq p < 0.10$), and ns = not significant.

Conversely, the GJ group showed higher levels of total PUFAs ($p < 0.001$), including both n-6 and n-3 series. These findings are consistent with previous literature reporting reduced muscular fat deposition together with a lower proportion of SFA and a higher proportion of PUFAs [47]. Notably, the increase was particularly evident in long-chain PUFAs (LC-PUFAs) of both the n-6 and n-3 series ($p < 0.001$). In contrast, precursor fatty acids such as linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) showed only trends toward significance ($p = 0.069$ and $p = 0.073$, respectively). This pattern may reflect reduced oxidative degradation of LC-PUFAs, potentially associated with the antioxidant activity of the GJ diet [15,18,47].

As shown in Table S3, among long-chain polyunsaturated fatty acids (≥C20), arachidonic acid (20:4 n-6, ARA) increased significantly in the GJ group, from 3.33% to 4.76% of total FAMEs ($p < 0.001$), along with its elongation product 22:4 n-6 (0.31% vs. 0.54%, $p < 0.001$), indicating a clear enrichment of long-chain n-6 derivatives within muscle tissue. Within the n-3 series, docosapentaenoic acid (22:5 n-3, DPA) showed the most pronounced increase, nearly doubling (0.52% vs. 1.04%, $p < 0.001$). Eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) also increased, although their absolute concentrations remained low in rabbit meat, as typically observed in terrestrial species [48]. The fatty acid profile expressed in absolute terms (Table S4) confirmed the main trends observed on a percentage of FAMEs, including the increase in long-chain n-3 and n-6 PUFAs, particularly in the n-3 fraction, as well as the reduction in saturated fatty acids. However, expressing the data in absolute terms resulted in greater variability, leading to the loss of statistical significance for some parameters. This is likely related to the low and comparable total lipid content of the muscle between groups.

Long-chain PUFAs derived from essential precursors (linoleic and α-linolenic acids) are of high nutritional importance, as humans possess a limited capacity for endogenous conversion and therefore rely partially on the dietary intake of preformed LC-PUFAs [49]. LC-PUFAs serve as precursors of eicosanoids such as prostaglandins and leukotrienes, regulate inflammatory responses, and are fundamental structural components of membrane phospholipids [50]. In particular, DPA has gained recognition as a biologically active intermediate between EPA and DHA, with potential roles in cardiovascular health and inflammatory regulation. Consequently, the enrichment observed in the GJ group may contribute to enhancing the nutritional value of rabbit meat [48].

The lower SFA proportion and the higher PUFA content observed in the GJ group may be associated with the modulation of hepatic lipid metabolism. The liver is the primary site for the desaturation and elongation of essential fatty acids in mammals, processes mediated by Δ5- and Δ6-desaturases (FADS1 and FADS2) and elongases of the ELOVL family [51]. Evidence indicates that hepatic oxidative stress can impair desaturase and elongase activities, thereby altering long-chain PUFA biosynthesis [18].

The n-6/n-3 ratio was significantly reduced in the GJ group compared with CN group (7.74 vs. 9.43; $p < 0.001$). From a human nutrition perspective, lowering the n-6/n-3 ratio is considered beneficial, as higher n-6 relative to n-3 fatty acid intake has been associated with enhanced inflammatory processes and increased cardiovascular risk [52]. Rabbit meat is typically characterized by relatively elevated n-6/n-3 ratios [53], largely due to cereal-based feeding systems rich in linoleic acid, which favour tissue accumulation of n-6 PUFAs. In this context, the significant shift observed in the GJ group reflects an enhancement in the nutritional quality of the meat.

3.2.3. Nutritional and Metabolic Indices Obtained from Fatty Acids of LTL Muscle

Additional nutritional indices derived from the fatty acid profile were used to further evaluate lipid quality. These indices summarize the balance between fatty acids generally regarded as less favourable from a cardiovascular and metabolic perspective, mainly specific SFA associated with higher LDL-cholesterol, and those considered more favourable, particularly MUFAs and PUFAs, which may counterbalance these effects [54].

Among the various indices proposed in the literature, the AI and TI, originally introduced by Ulbricht and Southgate [35], remain widely used to characterize foods in terms of their lipid nutritional quality. The applicability of these indices across different food categories and their relevance to human health have been discussed by Chen et al. [54]. In the present study, the GJ diet significantly reduced ($p < 0.001$) both AI (0.76 vs. 0.65 for CN and GJ) and TI (1.17 vs. 1.01 for CN and GJ, respectively), indicating a shift toward a more cardioprotective lipid profile in rabbits receiving goji supplementation (Figure 2). The observed values remained within the ranges previously reported for rabbit meat [53,55], namely from 0.37 to 1.25 for AI, and from 0.40 to 1.47 for TI. These findings confirm that dietary strategies incorporating ingredients rich in bioactive compounds can improve the functional nutritional value of meat.

The peroxidation index (PI) reflects the theoretical susceptibility of lipids to oxidative deterioration as a function of fatty acid unsaturation, assigning progressively greater weight to fatty acids with increasing numbers of double bonds [36]. Accordingly, the higher PI observed in the GJ group ($p < 0.001$) can be attributed to the increased proportion of long-chain n-6 and n-3 PUFAs. Comparable increases in PI have been reported in rabbit meat following dietary supplementation with PUFA-rich ingredients, such as linseed, which promote the incorporation of long-chain PUFAs into tissue lipids [55,56]. Product-to-precursor ratios were used as compositional proxies of apparent desaturase and elongase activities (Table 5), and should be interpreted cautiously, especially for fatty acids present

in trace amounts [37]. Metabolic indices calculated from the fatty acid profile of LTL muscle were affected by goji supplementation (Table 5). Specifically, the Δ9 n-7 index (16:1n-7/16:0) was significantly lower in the GJ group compared with CN (1.09 vs. 1.25; $p = 0.049$), whereas the Δ9 n-9 index (18:1n-9/18:0) did not differ between treatments (3.69 vs. 3.65; $p = 0.722$). As both ratios are commonly used as compositional proxies of stearoyl-CoA desaturase (SCD) activity [16,37], this pattern indicates a selective modulation of the C16 pathway, while the C18 pathway remained unchanged. The reduction in Δ9 n-7 is consistent with a lower contribution of de novo lipogenesis in rabbits fed the GJ diet. It is important to note that rabbit muscle contains relatively low intramuscular lipid levels and exhibits a fatty acid profile inherently richer in PUFAs and poorer in MUFAs compared with other monogastric species [26]. Moreover, the central role of the liver in rabbit fatty acid metabolism, as emphasized by Zubiri-Gaitán et al. [47], supports the hypothesis that hepatic modulation, potentially linked to the hepatoprotective effects of goji, can translate into changes in muscle fatty acid composition.

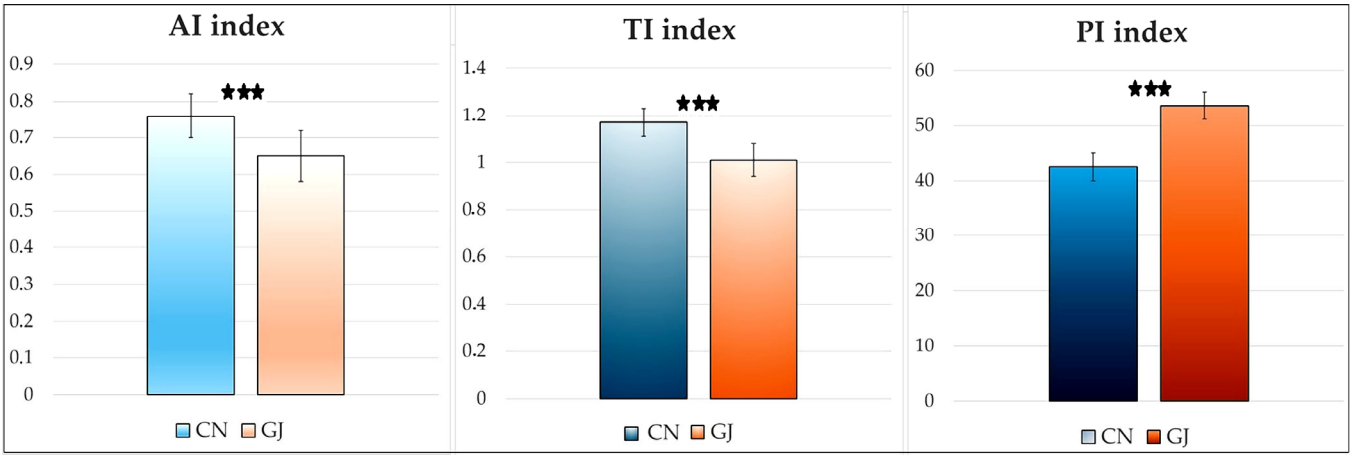

Figure 2. Nutritional indices calculated from fatty acids in *Longissimus thoracis et lumborum* muscle of rabbits fed two experimental diets. AI = atherogenic index, (12:0 + 4 × 14:0 + 16:0)/(∑ MUFA + ∑ PUFA); TI = thrombogenic index, (14:0 + 16:0 + 18:0)/(0.5 × ∑ MUFA + 0.5 × ∑ n-6 PUFA + 3 × ∑ n-3PUFA + n-3/n-6); PI = peroxidation index (0.025 × ∑ monoenoic acids%) + (∑ dienoic acids%) + (2 × ∑ trienoic acids%) + (4 × ∑ tetraenoic acids%) + (6 × ∑ pentaenoic acids%) + (8 × ∑ hexaenoic acids%). CN= control diet; GJ= CN + 3% of dried goji berries; values are expressed as mean ± standard deviation; statistical significance is indicated as *** $p < 0.001$.

Table 5. Metabolic indices calculated by fatty acid in *Longissimus thoracis et lumborum* of rabbits fed two experimental diets.

	CN	GJ	*Significance*
Δ9 n-7	1.25 ± 0.16	1.09 ± 0.20	*
Δ9 n-9	3.65 ± 0.33	3.69 ± 0.37	ns
Δ6 n-6	0.47 ± 0.07	0.50 ± 0.08	ns
Δ6 n-3	0.29 ± 0.06	0.34 ± 0.07	*
Δ5 n-6	18.37 ± 2.77	21.76 ± 2.96	**
Δ5 n-3	0.28 ± 0.03	0.32 ± 0.08	ns
ELOVL6	0.23 ± 0.01	0.24 ± 0.03	ns
ELOVL2–5 n-6	0.97 ± 0.22	1.13 ± 0.27	*
ELOVL2–5 n-3	5.10 ± 0.92	7.32 ± 2.04	***

For desaturase activity: Δ9 n-7 = 16:1n-7/16:0 × 10; Δ9 n-9 = 18:1 n-9/18:0; Δ6 n-6 = 18:3 n-6/18:2 n-6 × 100; Δ6 n-3 = 18:4 n-3/18:3 n-3; Δ5 n-6 = 20:4 n-6/20:3 n-6; Δ5 n-3 = 20:5 n-3/20:4 n-3 [37]. For elongate activity: ELOVL6 = 18:0/16:0; ELOVL2–5 n-6 = 22:4 n-6/20:4 n-6 × 10; ELOVL2–5 n-3 = 22:5 n-3/20:5 n-3 [38]. CN = control diet; GJ = CN + 3% dried goji berries; values are expressed as mean ± standard deviation; statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant.

Desaturases catalyze the introduction of double bonds at specific positions along the fatty acyl chain, counted from the carboxyl end, thereby increasing the degree of unsaturation of existing polyunsaturated fatty acids [50]. The Δ6 and Δ5 desaturation indices revealed selective differences between treatments (Table 5). While Δ6 n-6 did not differ between groups (0.47 vs. 0.50; $p = 0.625$), Δ6 n-3 was significantly higher in the GJ group (0.34 vs. 0.29; $p = 0.020$). A more pronounced effect was observed for Δ5 n-6, which increased from 18.37 to 21.76 ($p = 0.002$), whereas Δ5 n-3 showed only a non-significant upward trend (0.32 vs. 0.28; $p = 0.114$). Since Δ6 and Δ5 ratios are commonly used as compositional proxies of FADS2 and FADS1 activities, respectively, these results suggest a preferential modulation of long-chain PUFA metabolism, particularly within the n-6 pathway [37,57,58]. The marked increase in Δ5 n-6 is consistent with the higher proportion of arachidonic acid (20:4 n-6) observed in the GJ group (Table S3). Similarly, the increase in Δ6 n-3 aligns with the greater accumulation of long-chain n-3 derivatives. Importantly, the increase in long-chain PUFAs observed in the GJ group may not exclusively reflect enhanced enzymatic conversion. Highly unsaturated fatty acids are particularly susceptible to oxidative degradation; thus, the improved oxidative stability in the GJ group may have contributed to a greater preservation of long-chain PUFAs within muscle tissues [7,10,13]. This dual mechanism (modulation of metabolic flux and improved preservation) provides a coherent explanation for the enrichment of terminal derivatives such as 20:4 n-6 and 22:5 n-3.

Elongases (ELOVL enzymes) catalyze the extension of fatty acyl chains by adding two-carbon units at the carboxyl end, enabling the progressive synthesis of long-chain fatty acids from shorter precursors. In this context, the elongation indices further supported a selective modulation of long-chain PUFA metabolism [57,59]. ELOVL6 (18:0/16:0), reflecting the primary elongation step from palmitic to stearic acid, did not differ between treatments (0.23 vs. 0.24; $p = 0.298$). In contrast, both ELOVL2–5 n-6 and ELOVL2–5 n-3 were significantly higher in the GJ group. ELOVL2–5 n-6 increased from 0.97 to 1.13 ($p = 0.035$), while ELOVL2–5 n-3 showed a marked rise from 5.10 to 7.32 ($p = 0.001$). These indices, calculated as 22:4 n-6/20:4 n-6 and 22:5 n-3/20:5 n-3, reflect elongation steps leading to 22-carbon PUFAs. The observed increases are coherent with the higher proportions of 22:4 n-6 and 22:5 n-3 previously described in the GJ group.

Although ratio-based indices are compositional proxies and do not directly measure enzyme expression or activity, this pattern is consistent with the integrated action of desaturases and elongases in long-chain PUFA biosynthesis and may reflect metabolic modulation favouring LC-PUFA preservation and remodelling rather than isolated changes in single enzymes [38,57,59].

3.3. Shelf Life of Meat During Storage

3.3.1. Physical Quality Parameters During Storage

Water activity (a_w) and pH values measured in the LTL muscle remained stable throughout the storage period under both refrigerated (4 °C) and frozen (−20 °C) conditions (Table S5). No significant effects of diet, storage time, or their interactions were detected for either parameter ($p > 0.05$). Overall, pH values remained within the typical range for rabbit meat (≈5.74), while a_w values were consistently high (≈0.976), indicating that dietary goji supplementation did not influence these physicochemical traits during storage.

In contrast, storage time significantly influenced several colour parameters (Figure 3). During refrigerated storage (4 °C), lightness (L*) increased slightly over time in both dietary groups, reaching the highest values at 10 T (from 49.09 to 51.61 in CN, and from 51.54 to 52.63 in GJ). Yellowness (b*) decreased significantly between 1 T and 4 T ($p < 0.001$) and then remained relatively stable until 10 T. Hue angle remained stable in the CN group,

while a moderate increase was observed in the GJ group between 1 T and 4 T. Redness (a*, −2.54 on average) and chroma (3.22 on average) did not show significant variations during storage ($p > 0.05$). Changes in colour during refrigerated storage in rabbit meat are generally attributed to structural modifications of muscle tissue, variations in surface moisture, and progressive oxidative reactions affecting muscle pigments. The increase in L* observed in the present study is consistent with previous observations in rabbit meat during storage [56,60] and may be partially associated with the concomitant increase in drip loss, which enhances surface water and light reflectance [61].

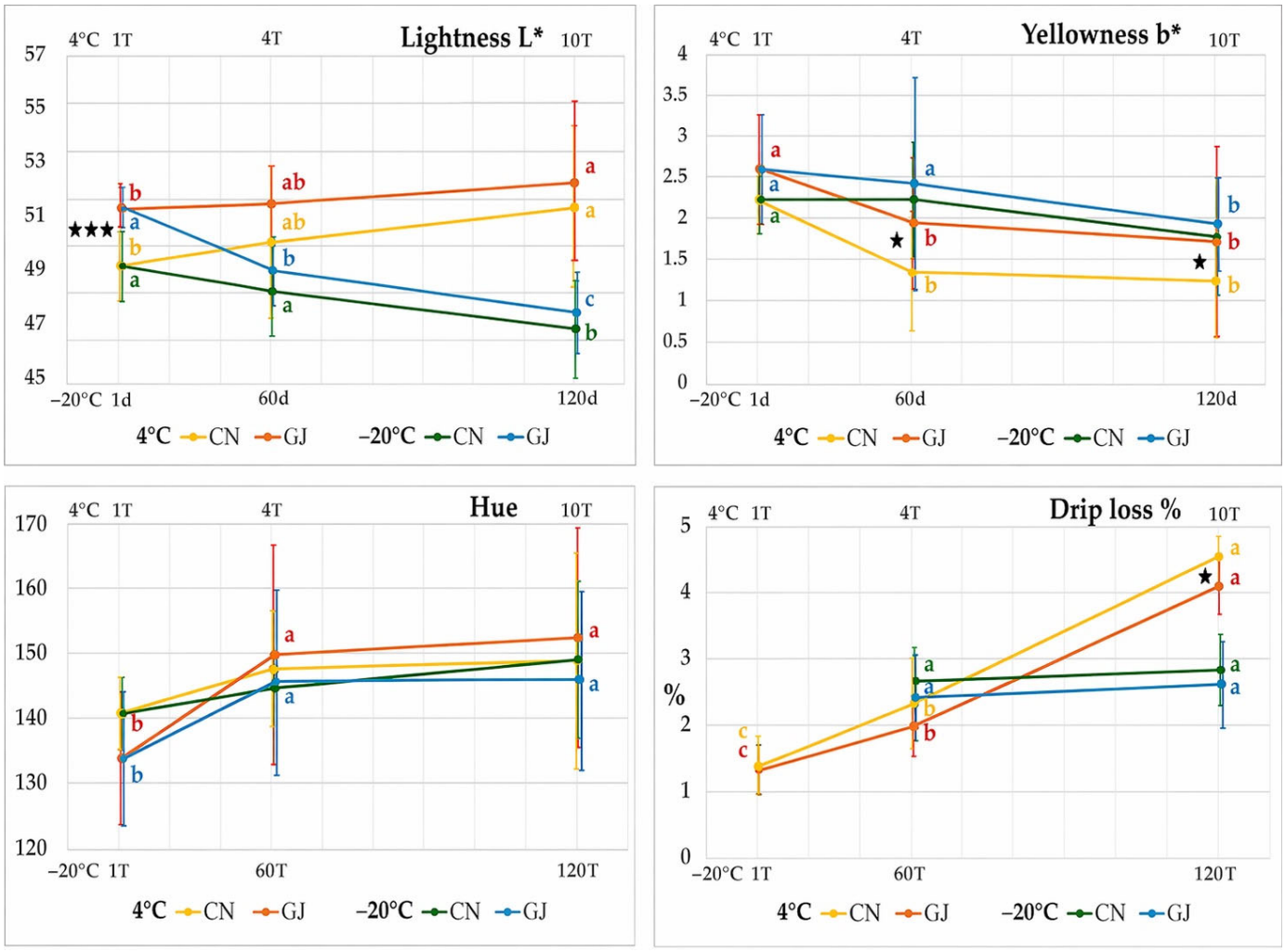

Figure 3. Effect of storage temperature and time on colour parameters (Lightness—L*, yellowness—b*, and hue angle), drip loss and thaw loss % in *Longissimus thoracis et lumborum* muscle of rabbits fed two experimental diets CN = control diet; GJ = CN + 3% dried goji berries; values are expressed as mean ± standard deviation. Different lowercase letters (a–c) indicate significant differences among storage times within the same treatment and temperature ($p < 0.05$); asterisks (*, ***) indicate significant differences between CN and GJ within the same storage condition and time (* $p < 0.05$, *** $p < 0.001$).

Under frozen storage (−20 °C), L* progressively decreased over time in both dietary groups, reaching the lowest values after 120 days. b* remained relatively stable in the CN group, whereas a significant reduction was observed in the GJ group at 120 T. In contrast, a* and chroma remained essentially unchanged throughout frozen storage in both groups. These results suggest that freezing induced structural changes in the muscle tissue that altered light reflectance without markedly affecting the stability of the red pigment

component [61]. In contrast to our findings, Villegas-Cayllahua [62] reported an increase in yellowness in rabbit meatballs stored at −18 °C for 120 days, likely associated with enhanced lipid oxidation. Dietary treatment exerted only minor effects on colour traits. Under refrigerated conditions, the GJ group exhibited higher L* at 1 T ($p = 0.002$) and higher b* values at 4 T and 10 T compared with the CN group, possibly due to the high carotenoid content of the GJ diet [63].

Drip loss increased significantly with storage time ($p < 0.001$). At the longest storage time, meat from the GJ group showed lower drip loss compared with the CN group (4.10% vs. 4.52% for GJ and CN, respectively; $p = 0.004$). During frozen storage, thaw loss remained relatively stable from 60 T until 120 T. These findings indicate that storage conditions were the primary factor influencing water loss [64].

Colour differences were expressed as ΔE values calculated in the CIELAB colour space from variations in L*, a*, and b* coordinates (Table 6). According to commonly adopted perceptibility thresholds, ΔE values greater than 3 are generally considered clearly perceptible to the human eye, ΔE values between 1.5 and 3 correspond to noticeable colour differences, and ΔE values below 1.5 are typically associated with minor or instrument-related variations [65]. During the early storage phase (1 T–4 T) under refrigerated conditions, ΔE showed a greater chromatic shift in the CN group (3.54) compared with the GJ group (1.90), whereas in prolonged refrigerated storage (1 T–10 T), ΔE values exceeded 3 in both groups, indicating clearly perceptible colour modifications, although no significant dietary effect was detected at this stage. The intermediate interval (4 T–10 T) showed more limited chromatic variation. A comparable effect was reported by Castrica et al. [23], who observed improved colour stability in rabbit meat from goji-supplemented animals during refrigerated storage, highlighting the potential role of goji bioactive compounds in preserving visual quality over time.

Table 6. Colour difference (ΔE) in the *Longissimus thoracis et lumborum* muscle during storage time within each dietary group and between groups at the same storage interval.

	ΔE 1 T–4 T	**ΔE 1 T–10 T**	**ΔE 4 T–10 T**
CN	3.54 ± 1.66 ^{a}	4.08 ± 1.70 ^{a}	2.21 ± 1.24 ^{b}
GJ	1.90 ± 0.73 ^{b}	3.55 ± 1.89 ^{a}	2.50 ± 1.74 ^{ab}
Significance between diets	**	ns	ns
	ΔE 1 T–60 T	**ΔE 1 T–120 T**	**ΔE 60 T–120 T**
CN	2.43 ± 1.23	3.31 ± 1.99	2.66 ± 1.70
GJ	3.39 ± 1.61 ^{ab}	4.76 ± 1.57 ^{a}	2.70 ± 1.45 ^{b}
Significance between diets	ns $^{¥}$	*	ns

ΔE was calculated as $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$; Differences in visually perceptible colour were classified as clearly perceptible (ΔE > 3), noticeable (1.5 < ΔE < 3), and minor or instrument-related (ΔE < 1.5) according to Patare et al. [65]. CN = control diet; GJ = CN + 3% dried goji berries. Values are expressed as mean ±standard deviation. Different letters within the same row indicate significant differences among storage intervals ($p < 0.05$). Significance between diets is reported for each storage interval as follows: * $p < 0.05$, ** $p < 0.01$, $^{¥}$ indicates a tendency ($0.05 \leq p < 0.10$), and ns = not significant.

For samples stored at −20 °C, the largest colour variation was observed between 1 T and 120 T, with ΔE values of 3.31 and 4.76 in CN and GJ, respectively, showing a significant difference between groups. Overall, ΔE values indicate that storage time exerted a significant effect on colour stability, whereas dietary treatment influenced visually perceptible colour differences only at specific storage intervals. These physical changes occurring during storage may interact with oxidative and degradative processes involving microbial spoilage, muscle lipids, and proteins [66].

3.3.2. Microbiological Results in *Longissimus Thoracis et Lumborum* Raw Meat During Storage

The microbiological status of meat at slaughter represents a key determinant of its subsequent shelf life. This initial microbial load depends on several factors, including animal physiology, slaughter hygiene, and post-mortem handling [67]. Previous studies have reported that the dominant microorganisms in meat stored under aerobic conditions are generally *Pseudomonas* spp., which are commonly associated with slime formation and off-odour development when present at levels of 7–8 $\log_{10}$ CFU/g [68,69]. When the atmosphere surrounding the meat contains both CO_2 and O_2, the growth of *Brochothrix thermosphacta* and lactic acid bacteria can also be observed [70]. Table 7 reports data related to the microbiological quality of rabbit meat from the CN and GJ groups stored at two different temperatures.

Table 7. Viable plate counts of microbial populations observed in samples stored at 4 °C and –20 °C, expressed as $\log_{10}$ CFU/g ± SD.

Diet	Time	TVC	*Pseudomonas* spp.	*Brochothrix thermosphacta*	*Lactobacillus* spp.	*Enterobacteriaceae*
CN	1 T	3.01 ± 0.52 ^{c}	1.87 ± 0.62 ^{c}	<DL *	1.82 ± 0.46	<DL *
	4 T	4.79 ± 0.53 ^{b}	4.42 ± 0.81 ^{b}	2.96 ± 0.64 ^{b}	1.85 ± 0.49	2.17 ± 0.73 ^{b}
	10 T	8.96 ± 0.15 ^{a}	9.15 ± 0.29 ^{aA}	6.72 ± 0.94 ^{a}	2.32 ± 0.32 ^{B}	4.41 ± 0.97 ^{a}
GJ	1 T	2.80 ± 0.55 ^{c}	2.31 ± 0.75 ^{b}	1.39 ± 0.89 ^{b}	1.91 ± 0.60 ^{b}	1.73 ± 0.17 ^{b}
	4 T	4.72 ± 1.09 ^{b}	3.86 ± 1.94 ^{b}	3.23 ± 1.52 ^{b}	2.78 ± 0.84 ^{b}	1.76 ± 0.36 ^{b}
	10 T	7.96 ± 1.39 ^{a}	6.29 ± 2.96 ^{aB}	6.47 ± 1.74 ^{a}	4.30 ± 1.85 ^{aA}	4.84 ± 1.20 ^{a}
Sign. between diets		ns	***	ns ¥	*	ns
Sign. among storage times		***	***	***	**	*
Sign. interaction		ns	***	*	**	*
CN	1 T	3.01 ± 0.52	1.87 ± 0.62	<DL *	1.82 ± 0.46 ^{a}	<DL
	60 T	2.48 ± 0.61	1.74 ± 0.55	1.35 ± 0.37 ^{b}	1.51 ± 0.32 ^{b}	<DL
	120 T	2.27 ± 0.50	1.59 ± 0.58	1.50 ± 0.28 ^{a}	1.53 ± 0.39 ^{ab}	<DL
GJ	1 T	2.80 ± 0.54 ^{a}	2.31 ± 0.75	1.39 ± 0.89	1.91 ± 0.60 ^{a}	1.73 ± 0.17
	60 T	2.27 ± 0.57 ^{ab}	2.41 ± 0.49	1.76 ± 0.66	1.16 ± 0.22 ^{b}	1.26 ± 0.45
	120 T	1.92 ± 1.00 ^{b}	2.19 ± 0.38	1.87 ± 0.54	1.42 ± 0.44 ^{b}	1.37 ± 0.43
Sign. between diets		ns	ns	*	*	ns ¥
Sign. among storage times		*	ns	ns	***	ns
Sign. interaction		*	ns	*	*	ns

Values < DL were assigned 0.5 $\log_{10}$ CFU/g (half the DL) for analysis; DL: detection limit (* 1 $\log_{10}$ CFU/g). TVC: total viable counts; CN = control diet; GJ = CN + 3% dried goji berries. Different lowercase letters within columns indicate significant differences among storage times within the same dietary group ($p < 0.05$); different uppercase letters among diets within the same time point indicate significant differences at ($p < 0.05$). Data are expressed as mean ± standard deviation; statistical significance is indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ¥ indicates a tendency ($0.05 \leq p < 0.10$); ns = not significant.

During refrigerated storage, both diet and storage time influenced the overall microbiological quality of the meat. In particular, storage time significantly ($p < 0.001$) affected TVC, *Pseudomonas*, and *Brochothrix thermosphacta* populations, and, to a lesser extent, *Lactobacillus* spp. ($p < 0.05$). *Pseudomonas* counts were also significantly affected by the dietary treatment ($p < 0.001$). In detail, all samples displayed a gradual increase in total viable counts (TVC) from day 1 (1 T) to day 10 (10 T) in both dietary groups. According to other authors [71], similar aerobic mesophilic counts were observed for 14 days refrigerated storage (4 °C) (from 2.22 ± 0.57 to 5.66 ± 0.49 $\log_{10}$ CFU/g) of raw aerobically packed LTL rabbit meat. *Pseudomonas* spp., recognized as the dominant spoilage microorganisms under aerobic conditions, increased over time in both groups; however, a markedly lower growth was observed in the GJ group compared with the CN group at 10 T (9.15 vs. 6.29 log CFU/g, corresponding to ~2.9 log difference). This substantial difference is relevant from a

practical perspective and suggests a potential inhibitory effect associated with dietary goji supplementation on this specific microbial population, potentially extending the meat's shelf life. Conversely, *Lactobacillus* spp. populations increased progressively in GJ samples and remained higher than in CN samples at day 10. This pattern is consistent with findings by Castrica et al. [23] in meat from rabbits fed goji berries, who reported a positive role of *Lactobacillus* spp. in counteracting the development of undesirable bacteria on the meat surface, probably through competitive exclusion mechanisms, as widely documented in other studies [72]. However, the role of lactic acid bacteria in fresh meat remains debated. Some species produce metabolites that accelerate sensory deterioration, whereas other LAB strains act as bioprotective agents during storage by competitively inhibiting spoilage and pathogenic microorganisms [73,74]. *Brochothrix thermosphacta* exhibited similar growth patterns in both CN and GJ LTL samples, indicating no dietary effect on this psychrotrophic species, whose abundance increased over time. Likewise, *Enterobacteriaceae* counts were not significantly affected by the diet, although they increased during storage time in both groups. Total coliforms were not detected in any samples.

Frozen-stored samples showed limited microbial growth across all groups, and only minor changes in microbial populations were observed over time during storage. A significant effect of storage time was observed for *Lactobacillus* populations, whereas no significant changes were detected for *Pseudomonas* spp., *Brochothrix thermosphacta,* and *Enterobacteriaceae*. TVC declined during frozen storage from day 1 to day 120 in the GJ group. *Pseudomonas* spp. population counts indicated no temporal changes under frozen storage in either diet, and mean values remained low. *Lactobacillus* spp. at 60 T and 120 T in both groups were slightly lower than the initial levels observed at 1 T and displayed significant effect of time ($p < 0.001$). *Enterobacteriaceae* were not significantly affected by either diet or storage time. Coliforms were undetectable (<DL) both before and throughout storage in all samples. Overall, the GJ diet slightly improved the microbiological quality of the meat under both refrigerated and frozen storage conditions.

3.3.3. Chemical Analysis Results During Storage

TBARSs, TVB-N, and Thiol and Carbonyl Groups During Storage

Storage temperature and time markedly influenced oxidative and degradative processes in rabbit meat (Figure 4), with clear differences between refrigerated and frozen storage. Under refrigerated conditions (4 °C), lipid peroxidation increased progressively over time in both dietary groups, as evidenced by the significant rise in TBARS values ($p < 0.001$). At the beginning of storage (1 T), TBARS levels were extremely low (≈0.017 mg MDA/kg meat), indicating minimal lipid oxidation immediately after dissection. During storage, however, TBARS values increased markedly, reaching 0.33 mg MDA/kg in CN and 0.23 mg MDA/kg in GJ at 10 T, with significantly lower values in GJ meat ($p < 0.001$). The lower TBARS accumulation in the GJ samples indicates that dietary goji supplementation effectively delayed lipid oxidation during chilled storage. This result is particularly noteworthy considering that the fatty acid profile of the GJ meat showed a higher proportion of PUFAs and a significantly greater PI (Figure 2). From a biochemical perspective, PUFA enrichment generally increases the susceptibility of muscle lipids to oxidative deterioration because double bonds represent preferential sites for radical attack and propagation reactions [57]. On the other hand, the lowest TBARS content observed in the GJ group, despite its higher PI, suggests that antioxidant compounds and the presence of bioactive components supplied by goji berries were able to reduce lipid oxidation [10,11,15], as reported also by Castrica et al. [23]. Goji berries contain a wide range of bioactive compounds, including carotenoids (especially zeaxanthin), flavonoids, phenolic acids and polysaccharides, which are widely recognized for their antioxidant activity [11,13]. These molecules can act

through multiple mechanisms, including radical scavenging activity, interruption of lipid peroxidation chain reactions and chelation of pro-oxidant metal ions [75,76]. Under frozen storage (−20 °C), TBARS values remained very low throughout the experimental period and did not show the marked accumulation observed under refrigeration, remaining close to initial values (≈0.012–0.018 mg MDA/kg meat). This confirms that freezing strongly slows oxidative reactions by reducing molecular mobility, limiting oxygen diffusion, and suppressing enzymatic activity in the muscle matrix [77,78]. Consequently, under frozen conditions, the protective effect of dietary antioxidants becomes less evident, as oxidative processes themselves are already strongly inhibited by low temperature.

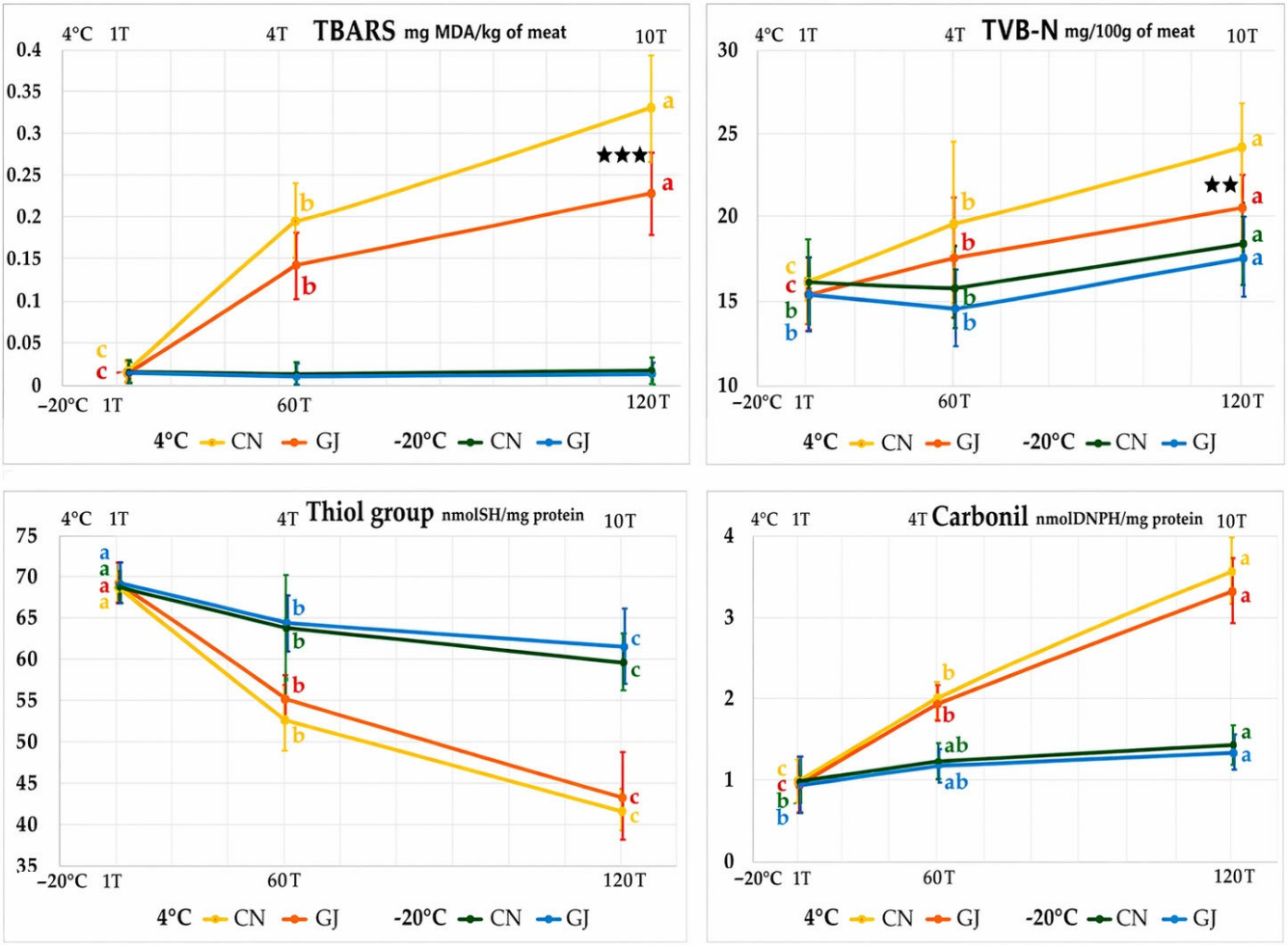

Figure 4. Effect of storage temperature and time on lipid and protein oxidation parameters in *Longissimus thoracis et lumborum* muscle of rabbits fed two experimental diets. Thiobarbituric acid reactive substances (TBARSs, mg MDA/kg meat), total volatile basic nitrogen (TVB-N, mg/100 g meat), thiol group content (SH, nmol/mg protein), and protein carbonyl content (DNPH, nmol/mg protein) were determined in samples stored at 4 °C at 1 (1 T), 4 (4 T), and 10 days (10 T) and −20 °C at 1 (1 T), 60 (60 T) and 120 days (120 T). CN = control diet; GJ = CN + 3% dried goji berries. Values are expressed as mean ± standard deviation. Different lowercase letters (a–c) indicate significant differences among storage times within the same treatment and storage condition ($p < 0.05$), whereas asterisks (**, ***) indicate significant differences between dietary treatments within the same storage time ($p < 0.01$ and $p < 0.001$, respectively).

A similar storage-dependent pattern was observed for total volatile basic nitrogen (TVB-N), which reflects the accumulation of volatile nitrogen compounds generated by protein degradation and microbial metabolism. During refrigerated storage, TVB-N increased

significantly in both dietary groups ($p < 0.001$), rising from approximately 16 mg/100 g meat at 1 T to about 24 mg/100 g in CN and 20 mg/100 g in GJ at 10 T. The significantly lower TVB-N values observed in the GJ group ($p < 0.01$) suggest that dietary goji supplementation may have delayed the progression of spoilage processes during chilled storage [79]. Although Castrica et al. [23] did not observe significant differences in TVB-N in rabbit meat supplemented with goji berries, the lower accumulation of volatile nitrogen compounds observed in the present study suggests that the antioxidant and bioactive compounds of goji may indirectly influence protein stability during storage. Under frozen storage, TVB-N values increased slowly, and significant differences were only observed after prolonged storage (120 T), reaching values around 18 mg/100 g meat, with no clear differences between dietary treatments. These results confirm the protective effect of frozen storage [77].

Protein oxidation markers confirmed that oxidative deterioration progressed during storage and was mainly driven by storage conditions rather than dietary treatment. During refrigerated storage, thiol group content decreased significantly ($p < 0.001$), while protein carbonyls increased from approximately 1.0 nmol DNPH/mg protein at 1 T to about 3.5 nmol/mg protein at 10 T. The progressive depletion of thiol groups observed during storage is likely related to the oxidation of cysteine residues [80]. These reactions are often accompanied by the formation of protein carbonyls, which represent stable markers of irreversible oxidative damage and are widely used to assess protein oxidation in biological systems. Under frozen conditions, both thiol depletion and carbonyl formation occurred at a much slower rate, confirming that low temperature effectively limits oxidative modifications in muscle proteins.

Biogenic Amines (BA) Content

Physiological polyamines—primarily putrescine, spermidine, and spermine—form a distinct group of biologically active molecules characterized by specific biosynthetic pathways and essential functions in cell growth and metabolism. Among free polyamines, spermidine and spermine are the most common and abundant in various food sources, including animal, plant-derived, and microbial sources, while putrescine and cadaverine are frequently found in fermented or spoiled food [81]. In contrast, several BAs such as cadaverine and histamine arise mainly from microbial decarboxylation of amino acids and are closely linked to food spoilage and safety concerns [4]. Because these compounds differ markedly in origin, biological roles, and toxicological relevance, their combined assessment provides valuable insights into both endogenous polyamine metabolism and microbial activity in food systems [5,82]. In particular, the BAs selected for investigation in this study were chosen because, as reported by Del Rio et al. [83], spermine, an endogenous antioxidant polyamine, was found to be 5.5-fold more cytotoxic than spermidine in a human intestinal in vitro model. Moreover, unlike spermidine, tyramine, putrescine, and cadaverine which appear to exert their cytotoxic effects mainly through necrosis [84], the biogenic amine histamine has been shown, in the same in vitro model, to induce cell death via an apoptotic mechanism [85]. Table 8 presents the concentrations of biogenic amines in rabbit meat from the CN and GJ groups stored under two different temperatures.

During refrigerated storage, cadaverine and histamine increased significantly over time in both dietary groups, reflecting the proteolytic activity typically associated with microbial spoilage, with storage time exerting a significant effect. A significant dietary effect ($p = 0.018$) indicated that GJ samples accumulated higher overall cadaverine concentrations than CN throughout storage. The GJ diet may induce alterations in the muscle amino acid profile, including an increased availability of free lysine—the limiting amino acid precursor for cadaverine formation—which may influence post-mortem proteolysis by modulating

the activity of endogenous and/or microbial proteases [86]. Thus, cadaverine accumulation was strongly dependent on storage time and modulated by diet under refrigerated storage conditions. Since BAs are formed through the decarboxylation of free amino acids (FAAs), and changes in the amino acid profile during meat storage arise from proteolysis—often driven by microbial activity—*Lactobacillus* spp. are known contributors to sensory deterioration in meat due to their proteolytic activity [87]. Our results support this hypothesis, as a significant increase in the *Lactobacillus* population was observed in the GJ group under refrigerated storage conditions (Table 7). Moreover, it is well known that not only spoilage bacteria (e.g., *Enterobacteriaceae*) but also several LAB are capable of producing cadaverine. In particular, *Lactobacillus* spp., such as *L. brevis*, *L. plantarum*, *L. curvatus* and *L. sakei*, possess lysine decarboxylase-like genes, supporting their ability to contribute to cadaverine accumulation [88]. Conversely, the diet effect was not significant ($p = 0.643$) for histamine, indicating that dietary treatment did not alter its overall production (Table 8). Unlike histamine, spermine concentration was influenced by both diet ($p = 0.032$) and storage time ($p < 0.001$). In both CN and GJ groups, spermine increased at 4 T and then decreased at 10 T, indicating a similar unstable temporal pattern in the two dietary treatments. The progressive decrease in spermine over time may be interpreted in the context of the marked oxidative processes occurring under refrigerated storage [81]. However, at the end of refrigerated storage (10 T), spermine concentration was lower in the GJ group than in the CN group, suggesting that dietary treatment may have influenced polyamine degradation during prolonged storage. Considering the recognized antioxidant role of spermine in muscle tissues. These findings are in agreement with Qiao et al. [81], who reported that polyamines may counteract oxidative processes; in our study, this was reflected by the lower accumulation of TBARSs (Figure 4). These authors reported that polyamines, particularly spermine, are known to act as free radical scavengers and membrane stabilizers and may therefore be consumed more rapidly under conditions of enhanced oxidative challenge or altered redox balance. Recently, Reséndiz-Cruz et al. [71] investigated nitrogenous compounds in rabbit meat stored under refrigerated conditions and aerobic packaging, as our condition. They found that cadaverine and histamine concentrations significantly increased after 7 days of storage, in agreement with our findings.

To the best of our current knowledge, the unexpected marked reduction in BAs (Table 8) during frozen storage should be interpreted with caution. Freezing may induce structural alterations in muscle tissue and/or affect BA extractability, potentially influencing analytical recovery. Alternatively, interactions between amines and denatured proteins cannot be excluded. These findings warrant further investigation.

The statistical analysis showed that time had a significant effect ($p < 0.001$) on cadaverine and histamine, whereas diet did not significantly influence any of the three studied biogenic amines. In particular, cadaverine in CN decreased from 33.43 mg/kg at 1 T to ~14 mg/kg at both 60 T and 120 T; in GJ, it also showed a substantial reduction, from 37.00 mg/kg at 1 T to 7.29 mg/kg at 60 T, followed by a moderate increase at 120 T (20.09 mg/kg). Histamine in CN samples decreased from 65.03 mg/kg at 1 T to 25.76 mg/kg at 120 T; in GJ it showed a similar reduction from 53.00 mg/kg to 31.37 mg/kg at 60 T, although a moderate increase occurred by 120 T (45.12 mg/kg). Spermine concentration remained largely unchanged during frozen storage regardless of dietary treatment. In CN samples, spermine decreased moderately from 63.87 mg/kg at 1 T to 45.92 mg/kg at 120 T whereas in GJ samples, it decreased from 56.40 mg/kg to 37.42 mg/kg over the same period. Although a decreasing trend was observed, these changes did not reach statistical significance, consistent with the relative chemical stability of endogenous polyamines under low-temperature conditions. Dadáková et al. [89] reported that spermine concentration decreased significantly in frozen-stored rabbit meat during the first 3 months, and

the decrease continued during a further 5 months, with relative losses of 30–35% of the initial concentration, comparable to the losses observed in our dietary groups (about 28% after 2 months of storage). Similar storage-related variations in polyamine concentrations have been reported in different types of meats, showing decreases [90], moderate increases [91], or an initial rise followed by a marked decline [92]. To our knowledge, there is no information on the mechanism of the polyamine losses during frozen storage.

Table 8. Biogenic amine concentrations in rabbit *Longissimus thoracis et lumborum* muscle from two dietary groups during refrigerated storage at 4 °C and −20 °C.

Diet	Time	Cadaverine mg/kg	Histamine mg/kg	Spermine mg/kg
CN	1 T	33.43 ± 3.30 ^{b}	65.03 ± 4.83 ^{b}	63.87 ± 2.75 ^{b}
	4 T	39.62 ± 2.68 ^{b}	75.68 ± 5.67 ^{b}	83.69 ± 5.68 ^{a}
	10 T	100.51 ± 6.17 ^{aB}	178.20 ± 8.51 ^{a}	61.71 ± 6.58 ^{bA}
GJ	1 T	37.00 ± 3.26 ^{b}	53.00 ± 4.28 ^{c}	56.40 ± 6.74 ^{b}
	4 T	53.30 ± 5.29 ^{b}	85.42 ± 5.10 ^{b}	88.53 ± 6.34 ^{a}
	10 T	150.36 ± 11.39 ^{aA}	185.29 ± 6.66 ^{a}	44.81 ± 3.96 ^{bB}
Sign. between diets		*	ns	*
Sign. among storage times		***	ns $^{¥}$	***
Sign. interaction		**	**	***
CN	1 T	33.43 ± 3.30 ^{a}	65.03 ± 4.83 ^{a}	63.87 ± 2.75
	60 T	14.54 ± 1.28 ^{b}	38.81 ± 4.77 ^{b}	56.46 ± 4.01
	120 T	14.27 ± 2.28 ^{b}	25.76 ± 3.36 ^{c}	45.92 ± 1.51
GJ	1 T	37.00 ± 3.26 ^{a}	53.00 ± 4.28 ^{a}	56.40 ± 6.74
	60 T	7.29 ± 1.21 ^{c}	31.37 ± 2.07 ^{c}	40.74 ± 1.88
	120 T	20.09 ± 1.97 ^{b}	45.12 ± 5.00 ^{b}	37.42 ± 6.32
Sign. between diets		ns	ns $^{¥}$	ns
Sign. among storage times		***	***	ns
Sign. interaction		*	**	ns

CN = control diet; GJ = CN + 3% dried goji berries. Different lowercase letters within columns indicate significant differences among storage times within the same dietary group ($p < 0.05$); different uppercase letters among diets within the same time point indicate significant differences at ($p < 0.05$). Data are expressed as mean ± standard deviation; statistical significance is indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $^{¥}$ indicates a tendency ($0.05 \leq p < 0.10$); ns = not significant.

3.3.4. Principal Component Analysis of Quality Parameters During Storage

The generalized PCA (Figure 5) provided an integrated overview of the relationships among physical, chemical, microbiological, and BA parameters during storage and clearly discriminated samples according to storage conditions and time.

Under refrigerated storage (4 °C), PC1 explained 77% of the total variance, indicating that storage time was the primary driver of quality changes. Samples at 1 day were distributed on the negative side of PC1 and were mainly associated with higher thiol group content and colour stability parameters (chroma), reflecting preserved protein integrity and limited oxidative damage at the early stage of storage. After 4 days of storage, samples shifted toward an intermediate position and were associated with spermine, suggesting partial maintenance of endogenous polyamines before the onset of marked degradative processes. By 10 days of refrigeration, samples were clearly separated along the positive axis of PC1 and strongly associated with TBARSs, carbonyls, TVC, drip loss, histamine, and cadaverine. This clustering confirms the progressive interaction between lipid oxidation, protein oxidation, microbial proliferation, and BA accumulation during prolonged chilled storage. The close alignment of TBARSs, carbonyls, TVC, histamine, and cadaverine

vectors further highlights the strong interdependence between oxidative deterioration and microbial spoilage under aerobic refrigeration.

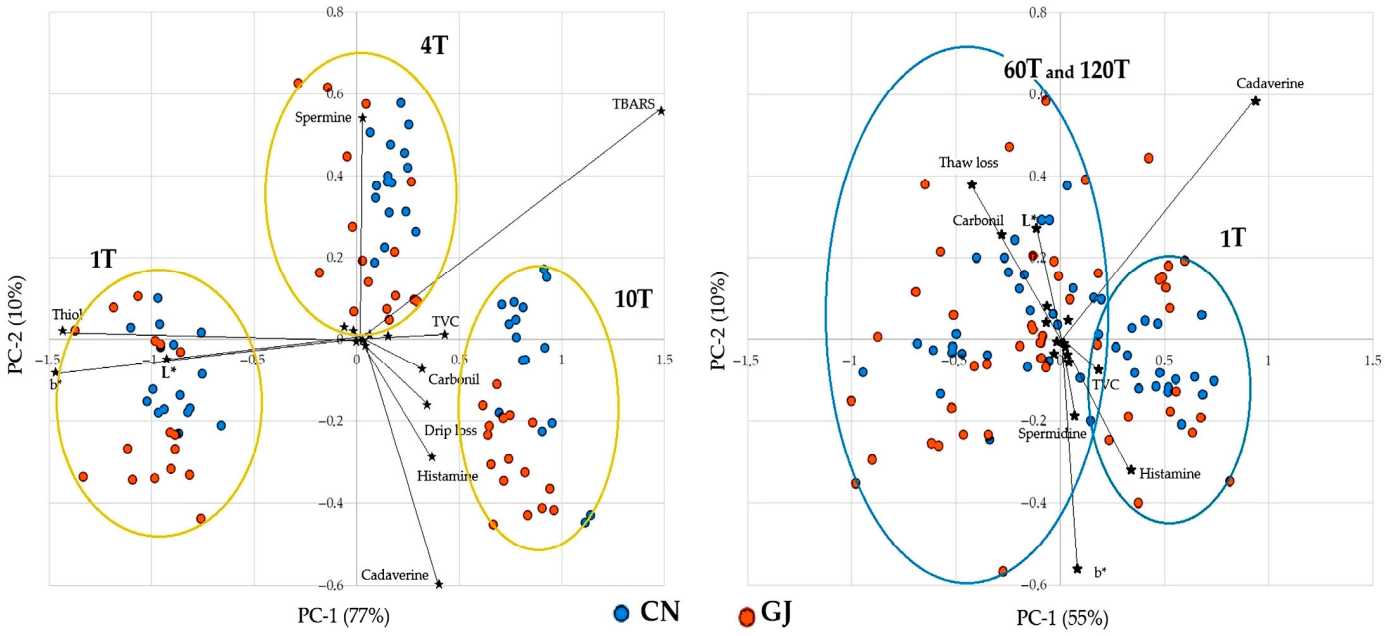

Figure 5. Generalized Principal Component Analysis (PCA) of physical (L*, a*, b*, chroma C*, hue angle, pH, a_W, drip loss, thaw loss), chemical (TBARSs, TVB-N, carbonyls, thiol groups, cadaverine, histamine, spermine), and microbiological (TVC) parameters during refrigerated and frozen storage of *Longissimus thoracis et lumborum* muscle from rabbits fed two experimental diets. CN = control diet; GJ = CN supplemented with 3% dried goji berries. The left panel shows samples stored at 4 °C (1, 4, and 10 days), whereas the right panel shows samples stored at −20 °C (1, 60, and 120 days). Score (CN blue points; GJ red points) and loading (variables) plots are presented together in the same biplot, and storage-time groups are delineated by ellipses. The percentage of variance explained by each PCA is reported alongside the axes for both models. Loading vectors represent the contribution of each variable to the principal components; longer vectors indicate a stronger contribution to the explained variance.

Within refrigerated storage, a partial separation between dietary groups was also observed. CN samples (blue) tended to be positioned closer to the vectors associated with oxidative and microbial spoilage markers, particularly at later storage times, whereas GJ samples (orange) were relatively shifted toward areas associated with preserved thiol content and lower oxidative load. Although storage time remained the dominant discriminating factor, this spatial distribution suggests a moderating effect of dietary goji supplementation on the progression of oxidative and spoilage-related changes.

In contrast, the inclusion of frozen samples (−20 °C) in the second PCA model resulted in a less pronounced temporal separation. Although PC1 explained 55% of the variance, the scores of samples stored for 60 and 120 days were more closely distributed, indicating that time under frozen conditions exerted a more limited influence on the overall multivariate profile compared with refrigeration. This pattern is consistent with the microbiological data, which showed stabilization or reduction in total viable counts and other microbial populations during frozen storage. Similarly, BAs did not exhibit the marked time-dependent increase observed at 4 °C. The relatively compact distribution of frozen samples in the score plot suggests that freezing mitigated the progression of spoilage-related processes, limiting divergence over time. It should be noted that the ellipses shown in the PCA plots are intended solely as a visual guide to illustrate the distribution of samples and do not represent statistically defined clusters. Loading vectors indicate that oxidative markers and

quality parameters still contributed to sample positioning under stored conditions. Overall, the PCA confirms that refrigeration promotes a coordinated progression of oxidative and microbiological deterioration, whereas freezing attenuates temporal variability and maintains a comparatively stable quality profile over extended storage.

4. Conclusions

Overall, the present study demonstrates that dietary supplementation with goji berries can simultaneously enhance the nutritional value and storage stability of rabbit meat. Goji inclusion improved the fatty acid profile by increasing long-chain PUFAs and reducing the n-6/n-3 ratio, while effectively limiting lipid peroxidation during refrigerated storage despite the higher degree of lipid unsaturation. The lower accumulation of TBARSs and volatile nitrogen compounds, together with reduced development of some spoilage-related microbial populations, indicates that the bioactive compounds naturally present in goji berries contribute to preserving meat quality during storage.

These findings highlight the potential of goji berries as a functional feed ingredient capable of improving both the technological stability and the nutritional profile of rabbit meat. In addition, the inclusion of phytochemical-rich feed ingredients may represent a promising strategy for the sustainable production of high-quality meat products. In this context, further research is warranted to validate these results under a broader range of conditions, including different forms of goji (fresh and dried), inclusion levels, rabbit genotypes, sexes, and production systems, in order to identify optimal supplementation strategies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app16094167/s1, Table S1: Chemical composition and percentage of fatty acids of the two experimental diets and dried Goji berries; Table S2: Principal bioactive compounds in Italian air-dried goji berries; Table S3: Fatty acids expressed as percentage of total FAMEs of *Longissimus thoracis et lumborum* muscle in rabbits fed two experimental diets; Table S4: Fatty acid profile expressed mg/100 g of *Longissimus thoracis et lumborum* muscle of rabbits fed two experimental diets Table S5: pH and a_w on LTL rabbit muscle from two dietary groups during refrigerated and frozen storage.

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Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials; further inquiries can be directed to the corresponding author.

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