



## Grape seed supplementation in growing rabbits: Effect on meat quality

Manuel Scerra<sup>a,\*</sup>, Francesco Foti<sup>a</sup>, Pasquale Caparra<sup>a</sup>, Matteo Bognanno<sup>a</sup>, Paolo Fortugno<sup>a</sup>, Domenico Viglianti<sup>a</sup>, Domenico Autolitano<sup>a</sup>, Guido Mangione<sup>b</sup>, Martino Musati<sup>b</sup>, Luigi Chies<sup>a</sup>

<sup>a</sup> University of Reggio Calabria, Dipartimento di Agraria, Produzioni Animali, Via dell'Università, 25, 89124 Reggio Calabria, Italy

<sup>b</sup> University of Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Via Santa Sofia 100, 95123 Catania, Italy

### ARTICLE INFO

#### Keywords:

Polyphenols  
Fatty acid  
Shelf-life  
Lipid oxidation  
By-products

### ABSTRACT

Grape seed supplementation in rabbit diet on meat quality and performance was investigated. The experiment lasted 57 days and was conducted with forty Hycole rabbits 35 days-old (bodyweight:  $802.6 \pm 2.69$  g) that were randomly divided into two groups and fed either a control diet (Control group) or the same diet of the control group in which 5 % of barley and 5 % of maize were replaced with 10 % of grape seed (GS group). The dietary treatment influenced final body weight, average daily gain and dry matter intake which were higher ( $P < 0.05$ ) in the GS group than in the control group. The addition of 10 % grape seed in rabbit diet increased the accumulation of intramuscular fat ( $P < 0.05$ ), the levels of C18:1 *cis*-9 ( $P < 0.01$ ) and of the total monounsaturated fatty acids ( $P < 0.01$ ), and tended to increase ( $P = 0.061$ ) the sum of *n*-6 PUFA in meat, whereas reduced the sum of *n*-3 PUFA ( $P < 0.05$ ), the levels of C22:5 *n*-3 ( $P < 0.05$ ) and C22:6 *n*-3 ( $P < 0.05$ ), leading to an increase ( $P < 0.01$ ) in the *n*-6 to *n*-3 ratio in GS group than in control group. Grape seed supplementation reduced TBARS values ( $P < 0.01$ ) and protected meat from lipid oxidation over time ( $P < 0.01$ ), demonstrating that supplementation of 10 % grape seeds in the rabbit diet could improve shelf-life in meat.

### 1. Introduction

More than 70 % of grapes worldwide are used in wine production, and for every ton of grapes processed during production, approximately 175–300 kg of waste, such as skins, seeds and stalks, are generated (Contreras et al., 2022). The wine industry is one of the most important industries in agriculture, and worldwide wine production in 2024 was 225.8 million hectoliters, with Italy and France among the largest producers (International Organization of Vine and Wine (OIV), 2025).

To date, one of the central points of bioeconomic policies is the sustainability of production, which focuses particularly on waste reduction through recycling and reuse (Merli et al., 2018), and precisely with this in mind, the main wine-producing countries are evaluating the possibility of recovering production waste by transforming them into resources. The reuse of grape by-products can be beneficial for many businesses because they are rich in phytochemicals (Abouelenein et al., 2023; Frank et al., 2020), some of which have been tested as antioxidants to extend the shelf life of food products (Dhagare et al., 2022) or to protect plants from pests (Contreras et al., 2022). Among antioxidants, polyphenols are found in grape by-products and are also more abundant in seeds (Abouelenein et al., 2023). Wine waste, such as grape seeds and

skins, are also an important source of unsaturated fatty acids (Abouelenein et al., 2023; Beres et al., 2017).

Never before has the use of lower-cost protein sources become essential for the sustenance of families, considering the continuous increases in the costs of raw materials and in general of foods intended for human consumption. Among them, rabbit meat can play an important role in human nutrition by providing high-quality protein at low cost (Nasr et al., 2022). However, in 2023, increases in rabbit meat prices of around 15 % were observed. Therefore, the use of unconventional feed to reduce feeding costs in the rabbit industry, without compromising growth and meat quality, can play an important role. In addition, natural antioxidants such as polyphenols and flavonoids are often found in unconventional feed and can improve the oxidative stability of meat (López-Andrés et al., 2013). This is important considering that rabbit meat is particularly popular among consumers due to its richness in polyunsaturated fatty acids (Vizzarri et al., 2017), highly susceptible to lipid oxidation (Bekhit et al., 2013).

Recently, some authors investigated the effect of grape seed (Hafsa & Hassan, 2022) or grape pomace (Bouzaida et al., 2021) on growth performance and oxidative stress in rabbits. In addition, some studies (Garcia et al., 2002) observed that grape seed meal, a by-product

\* Corresponding author.

E-mail address: [manuel.scerra@unirc.it](mailto:manuel.scerra@unirc.it) (M. Scerra).

<https://doi.org/10.1016/j.meatsci.2025.109843>

Received 20 November 2024; Received in revised form 28 April 2025; Accepted 1 May 2025

Available online 2 May 2025

0309-1740/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

obtained by extracting the oil from grape seed, supplemented up to 15 % in the rabbit diet has positive effects on the growth performance of the animals. However, very little information is available in the literature on the effects of supplementation of grape seed alone on rabbit meat quality. Therefore, this study evaluates the effect of partial substitution of cereals with 10 % grape seed on the quality of rabbit meat. We hypothesized that supplementing the rabbit diet with grape seed could improve meat quality, especially oxidative stability, without affecting growth performance.

## 2. Materials and methods

### 2.1. Animals and experimental diets

The experimental methods of this trial have been authorized by the Animal Welfare Committee of the University of Reggio Calabria (protocol No. 1214).

The experiment lasted 57 days and was conducted with 40 Hycole rabbits 35 days-old, with a mean weight of  $802.6 \pm 2.69$  g. Rabbits were single housed in wire cages. They were randomly divided into 2 groups of 20 animals each and fed, after an adaptation period of 7 days, either a control diet (Control group) or the same diet of the control group in which 5 % barley and 5 % maize were replaced with 10 % of grape seed (GS group, Table 1). All diets were provided ad libitum in pellet form through the feeders in the cages, and animals had free access to water. Seeds from “Nerello Calabrese” grape were provided by a family winery from Bianco (Reggio Calabria, Italy). Regarding the diet of the GS group, the grape seeds were finely ground and mixed with the other ingredients and pelleted. The daily feed intake of the animals was assessed every day, and the animals were weighed every 15 days to calculate the ADG (average daily gain).

After the rabbits received the experimental diets for 57 days (7 days of adaptation period and 50 days of full experimental trial), they were

**Table 1**  
Ingredients (% on DM basis) and chemical composition of the experimental diets.

|  | Control diet | GS diet | Grape seed |
|--|--------------|---------|------------|
| Barley   | 10           | 5       |            |
| Maize  | 10           | 5       |            |
| Wheat bran   | 28           | 28      |            |
| Soybean meal   | 10           | 10      |            |
| Alfalfa meal   | 40           | 40      |            |
| Grape seed   | –            | 10      |            |
| Vitamin mineral premix <sup>1</sup>                      | 2            | 2       |            |
| <i>Chemical composition</i>                              |              |         |            |
| Dry matter (DM), g/kg wet weight                         | 887          | 890     | 910        |
| Crude protein, g/kg DM                                   | 157          | 158     | 103        |
| Ether extract, g/kg DM                                   | 25.9         | 28.6    | 80.2       |
| Ash, g/kg DM   | 38.7         | 39.1    | 38.8       |
| NDF, g/kg DM   | 330          | 370     | 383        |
| Total extractable polyphenols, g TAE <sup>2</sup> /kg DM | 4.02         | 5.70    | 19.7       |
| Total extractable tannins, g TAE <sup>2</sup> /kg DM     | 0.82         | 2.25    | 4.89       |
| $\alpha$ -tocopherol, $\mu$ g/g DM                       | 51.2         | 41.3    | 20.1       |
| <i>FA profile (g/100 g of total fatty acid)</i>          |              |         |            |
| C10:0  | 0.75         | 0.25    | 0.15       |
| C12:0  | 0.00         | 0.06    | 0.22       |
| C14:0  | 0.45         | 0.72    | 0.80       |
| C16:0  | 19.4         | 17.5    | 16.9       |
| C18:0  | 3.82         | 3.34    | 3.57       |
| C18:1 n-9  | 17.5         | 19.8    | 24.5       |
| C18:2 n-6  | 44.8         | 48.5    | 49.7       |
| C18:3 n-3  | 6.41         | 4.48    | 2.79       |

<sup>1</sup> The mineral vitamin premix consisted of vitamins A = 6750 UI; vitamin D3 = 1000UI; vitamin E 2 mg; vitamin B12 0.01 mg; vitamin B1 1mg; folic acid 0.2 mg; D-pantotenic acid 5 mg; Co 0.05 mg; Mn 12.5 mg; Zn 15 mg; Mo 0.5 mg;

<sup>2</sup> Tannic acid equivalent.

stunned and sacrificed (without fasting). The carcasses (including the head but excluding the distal parts of the tail and legs and all viscera) were subsequently weighed and transported to the Animal Production Unit of the University of Reggio Calabria, and chilled at +4 °C for 24 h. The *longissimus thoracis et lumborum* (LTL) muscles were removed from each animal (left and right) and divided into 2 parts (fore and posterior parts). The fore part of the left LTL muscle was used to determine TBARS values (thiobarbituric-acid reactive substances) during 8 days of refrigerated storage at 4 °C. The remaining parts of the LTL muscles were stored at –20 °C until analysis. These samples were used subsequently to evaluate the proximate composition (posterior part of left LTL muscle), fatty acid profile (fore part of the right LTL muscle), and antioxidant vitamins of meat (posterior part of right LTL muscle).

### 2.2. Chemical analyses of feedstuffs and meat proximate analyses

The dry matter (DM), ether extract (EE), crude protein and ash content of experimental feeds were determined according to the AOAC methods (AOAC, 1995) and NDF (neutral detergent fiber) was determined following the procedures described by Van Soest et al. (1991). The fatty acid (FA) composition of the feeds was determined following the method reported by Gray et al. (1967). Total extractable polyphenols and tannins were assessed following the Folin-Ciocalteu method (modified by Luciano et al., 2017) and tocopherols were extracted from 200 mg of feed samples as described by Rufino-Moya et al. (2020).

AOAC methods (AOAC, 1995) were used to determine meat proximate analyses (crude protein, moisture, ash and ether extract).

### 2.3. Fatty acid analysis, cholesterol and fat-soluble antioxidant vitamins of meat

LTL muscle intramuscular fat was extracted and quantified following the procedures described by Folch et al. (1957). A 2:1 (v:v) chloroform:methanol solution was used for extracting intramuscular fat from 5 g of muscle. A 100-mg portion was methylated by adding 0.05 mL of 2 N methanolic potassium hydroxide and 1 mL of hexane (IUPAC, 1987), with nonanoic acid as the internal standard. Analysis was performed on a ThermoQuest gas chromatograph (GC, ThermoQuest, Milan, Italy) equipped with a high-polar fused silica column (100 m, i.d. 0.25 mm, film thickness 0.25  $\mu$ m; SP 24056; Supelco Inc., Bellefonte, PA) and flame ionization detector (FID). Condition of GC and FAME identification were performed as reported by Scerra et al. (2022). The formulas indicated by Ulbricht and Southgate (1991) were used to evaluate atherogenic and thrombogenic indexes. For cholesterol and antioxidant vitamins, meat samples were analyzed following the method reported by Natalello et al. (2022) using a UHPLC system.

### 2.4. Lipid oxidation

Lipid oxidation of meat during refrigerated storage was measured using three slices of meat (2 cm thick), covered with a film of PVC and stored at 4 °C in the dark for 2 h (day 0), 4 and 8 days. TBARS assay was evaluated on each meat sample at each day of storage (Siu & Draper, 1978). For this analysis, the meat (2.5 g) was homogenized with distilled water (12.5 mL). Subsequently to the homogenized sample, trichloroacetic acid (12.5 mL, 10 % w/v) was added, vigorously vortexed, and filtered (Whatman No.1). Four mL of filtrate was combined with aqueous thiobarbituric acid (1 mL of 0.06 M), subsequently incubated at 80 °C for 90 min (water bath). The absorbance (532 nm) was measured with a Shimadzu spectrophotometer (UV-1800, Shimadzu, Milan, Italy) and results were evaluated by comparison with a TEP (1,1,3,3-tetraethoxypropane) calibrate curve (expressed as mg of malonaldehyde/kg of meat).

## 2.5. Statistical analysis

The Minitab 19 statistical package (Minitab Inc., State College, PA) was used to analyze the data, considering the dietary experimental treatments as factors in ANOVA analysis and considering the single animals as statistical unit. The effect of experimental diet on proximate composition, fatty acid profile and animal performance were analyzed using one-way ANOVA, while a mixed model for repeat measures was used to analyze data of lipid oxidation, where the terms in the model were dietary treatment, monitoring time and their interaction as fixed factor and individual animal as random factor.

Using Tukey's multiple comparison test, differences between means were assessed. Significance was declared when  $P \leq 0.05$ , and trends were considered when  $0.05 < P \leq 0.10$ .

## 3. Results

### 3.1. Meat proximate composition and animal performance

Table 2 shows the growth performance of experimental animals. Rabbits from GS group had higher ( $P < 0.05$ ) final body weight, ADG and dry matter intake (DMI) compared to the control rabbits. However, the feed conversion ratio (FCR) tended to increase ( $P = 0.082$ ) with grape seed supplementation. The effects on carcass weight were not observed.

The grape seed supplementation increased the level of  $\alpha$ -tocopherol ( $P < 0.001$ ) and tended to increase the level of EE ( $P = 0.086$ ) in meat compared to control treatment, while no significant differences between treatments were observed for crude protein, moisture, and ash.

### 3.2. Fatty acids and oxidative stability of meat

The effects of diet on the individual FA of muscle and intramuscular fat (IMF) are shown in Table 3. Replacing part of the barley and maize with 10 % grape seed increased the IMF ( $P < 0.05$ ). The summation of saturated fatty acids (SFA) was not different between groups, whereas the level of monounsaturated fatty acids (MUFA) was higher ( $P < 0.01$ ) and that of polyunsaturated fatty acids (PUFA) tended to be higher ( $P = 0.059$ ) in rabbit meat from the GS group than in meat from the other group. The SFA profile of LTL muscle was not affected by the dietary supplementation of grape seed. However, GS meat had a tendentially higher level of C14:0 ( $P = 0.083$ ) and C16:0 ( $P = 0.078$ ) than control meat. Furthermore, the level of C18:0 tended to decrease ( $P = 0.092$ ) by

**Table 2**

Rabbit performance in vivo and chemical composition of muscle (g/100 g wet weight).

|   | Dietary treatment <sup>1</sup> |      | SEM <sup>6</sup> | P value |
|---|--------------------------------|------|------------------|---------|
|   | Control                        | GS   |                  |         |
| Final BW <sup>2</sup> , g                                     | 2835                           | 3067 | 78.6             | 0.027   |
| Carcass weight, g   | 1657                           | 1765 | 54.1             | 0.318   |
| Total DMI <sup>3</sup> , g/d                                  | 155                            | 182  | 4.60             | 0.037   |
| ADG <sup>4</sup> , g/d  | 35.6                           | 39.7 | 1.79             | 0.033   |
| FCR <sup>5</sup> , g DMI <sup>3</sup> /g ADG <sup>4</sup>     | 4.4                            | 4.6  | 0.40             | 0.082   |
| <i>Tocopherol and cholesterol, <math>\mu</math>g/g muscle</i> |                                |      |                  |         |
| $\alpha$ -tocopherol  | 0.56                           | 3.10 | 0.293            | 0.001   |
| Cholesterol   | 1.34                           | 1.39 | 0.032            | 0.488   |
| <i>Chemical composition</i>                                   |                                |      |                  |         |
| Moisture, g/kg meat   | 74.2                           | 74.5 | 0.193            | 0.711   |
| Crude protein, g/kg meat                                      | 22.1                           | 22.3 | 0.191            | 0.485   |
| Ether extract, g/kg meat                                      | 1.98                           | 2.52 | 0.112            | 0.086   |
| Ash, g/kg meat  | 2.22                           | 2.27 | 0.119            | 0.491   |

<sup>1</sup> Treatments were: only concentrate (control) or the same diet of control group in which the grape seed (GS group) replaced 10 % dry matter (DM) on the diet fed.

<sup>2</sup> BW=Body weight; <sup>3</sup>DMI = dry matter intake; <sup>4</sup>ADG = average daily gain; <sup>5</sup>FCR = feed conversion ratio; <sup>6</sup>SEM = standard error of means.

**Table 3**

Fatty acid composition of LTL muscle (mg/100 g of muscle) from rabbits fed 10 % grape seed.

| Item                                  | Dietary treatment |      |                  |         |
|---------------------------------------|-------------------|------|------------------|---------|
|                                       | Control           | GS   | SEM <sup>4</sup> | P value |
| Intramuscular fat, mg/100 g of muscle | 1788              | 2179 | 150              | 0.042   |
| C10:0                                 | 5.26              | 4.96 | 1.088            | 0.927   |
| C12:0                                 | 4.56              | 4.30 | 1.000            | 0.692   |
| C14:0                                 | 38.9              | 52.7 | 2.069            | 0.083   |
| C16:0                                 | 508               | 612  | 44.17            | 0.078   |
| C16:1 <i>cis</i> -9                   | 62.7              | 107  | 7.131            | 0.059   |
| C17:0                                 | 11.3              | 13.7 | 1.582            | 0.169   |
| C18:0                                 | 169               | 134  | 4.239            | 0.092   |
| C18:1 <i>cis</i> -9                   | 435               | 613  | 19.61            | 0.001   |
| C18:2 <i>cis</i> -9, <i>cis</i> -12   | 470               | 562  | 17.10            | 0.067   |
| C20:0                                 | 3.69              | 2.52 | 0.471            | 0.102   |
| C18:3 <i>n</i> -3                     | 34.6              | 28.6 | 1.550            | 0.123   |
| C20:2 <i>n</i> -6                     | 5.23              | 5.95 | 0.501            | 0.239   |
| C20:3 <i>n</i> -3                     | 7.36              | 2.06 | 0.303            | 0.090   |
| C20:4 <i>n</i> -6                     | 18.0              | 26.6 | 2.061            | 0.100   |
| C20:5 <i>n</i> -3                     | 1.52              | 0.58 | 0.189            | 0.072   |
| C22:5 <i>n</i> -3                     | 8.76              | 4.97 | 0.5231           | 0.042   |
| C22:6 <i>n</i> -3                     | 2.01              | 0.67 | 0.193            | 0.045   |
| C24:0                                 | 0.23              | 0.13 | 0.018            | 0.531   |
| C24:1 <i>cis</i> -9                   | 1.21              | 3.73 | 0.399            | 0.301   |
| $\sum$ SFA <sup>1</sup>               | 742               | 824  | 83.31            | 0.167   |
| $\sum$ MUFA <sup>1</sup>              | 498               | 723  | 22.90            | 0.003   |
| $\sum$ PUFA <sup>1</sup>              | 547               | 631  | 22.40            | 0.059   |
| $\sum$ <i>n</i> -3                    | 54.9              | 36.9 | 2.103            | 0.017   |
| $\sum$ <i>n</i> -6                    | 493               | 594  | 19.51            | 0.061   |
| <i>n</i> -6/ <i>n</i> -3              | 9.39              | 14.5 | 1.089            | 0.001   |
| Thrombogenic index <sup>2</sup>       | 1.09              | 1.04 | 0.059            | 0.101   |
| Atherogenic index <sup>3</sup>        | 0.64              | 0.61 | 0.055            | 0.106   |

<sup>1</sup> SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

<sup>2</sup> Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA *n*-6 + 3 PUFA *n*-3 + PUFA *n*-3/PUFA *n*-6).

<sup>3</sup> Atherogenic index: (C12:0 + 4\*C14:0 + C16:0)/(MUFA + PUFA *n*-6 + PUFA *n*-3).

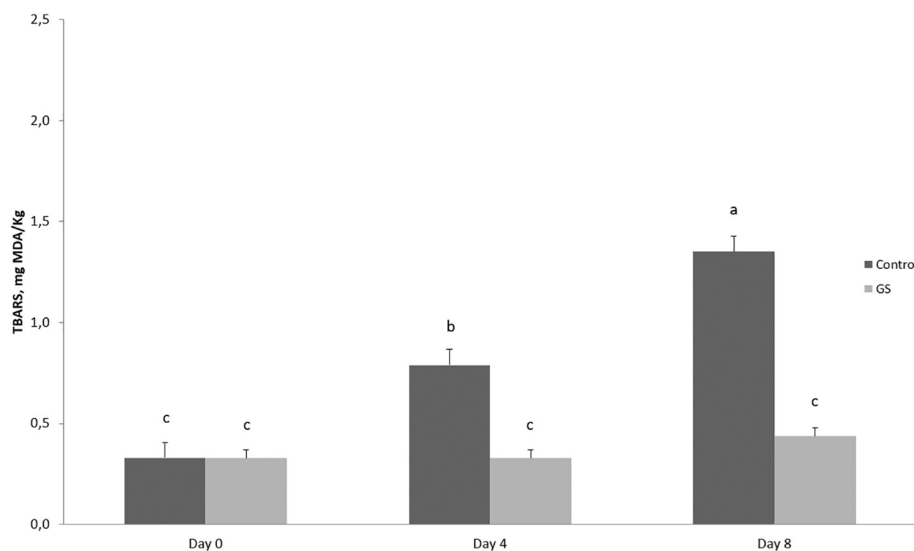
<sup>4</sup> SEM = standard error of means.

the administration of 10 % grape seed. Among the individual MUFA, the GS diet increased the concentration in muscle of oleic acid (C18:1 *cis*-9;  $P < 0.01$ ) and tended to increase the level of C16:1 *cis*-9 ( $P < 0.059$ ). The sum of *n*-3 PUFA was higher ( $P < 0.05$ ) in control meat than GS meat, whereas the sum of *n*-6 PUFA tended to be higher ( $P = 0.061$ ) in the meat from GS group than in the control meat. Consequently, the rabbit meat from the GS group had higher ( $P < 0.001$ ) value of the ratio *n*-6 to *n*-3 than the meat from the control group. However, the control group had higher ( $P < 0.05$ ) levels of docosapentaenoic acid (DPA, C22:5 *n*-3) and docosahexaenoic acid (DHA, C22:6 *n*-3) and showed value that tended to be higher of eicosapentaenoic acid (EPA, C20:5 *n*-3,  $P = 0.072$ ) in muscle than the GS group, whereas among the *n*-6 PUFA, the concentration of linoleic acid (C18:2 *cis*-9 *cis*-12) tended to increase by integrating grape seed ( $P = 0.067$ ).

Fig. 1 shown the results of the TBARS test during the 8 days of meat storage. Grape seed supplementation in the rabbit diet reduced TBARS values ( $P < 0.01$ ) and protected meat from lipid oxidation over time ( $P < 0.01$ ). The diet  $\times$  time interaction was significant ( $P < 0.001$ ). In meat from control group lipid oxidation linearly increased ( $P < 0.001$ ) over time, whereas in GS meat the TBARS values were comparable for all days of observation.

## 4. Discussion

In this trial, the main growth performance parameters were influenced by the integration of grape seed in the rabbit diet. In fact, the final body weight and the ADG of the rabbits in the GS group were higher than in the control group. Furthermore, the rabbits in the GS group ingested a greater quantity of feed compared to the animals in the



**Fig. 1.** Effect of grape seed supplementation and time of storage on lipid oxidation (TBARS assay) in meat slices over aerobic storage at 4 °C. Control, basal diet; GS, basal diet supplemented with 10 % of grape seed. TBARS, thiobarbituric acid reactive substances expressed as mg of Malondialdehyde (MDA) per kg of meat. Error bars represent the standard error of the mean. <sup>a,b,c</sup> Values with different superscripts are significantly different ( $P \leq 0.05$ ).

control group, 182 vs 155 g/d, respectively for GS and control groups. The higher DMI certainly influenced the higher ADG and final body weight of rabbits in the GS group. Similar results on final body weight and body weight gain were observed by other authors (Hafsa & Hassan, 2022) in New Zealand rabbits that received daily 50 g grape seed/kg diet fed. Even Hafsa and Ibrahim (2018) found higher ADG and higher final body weight in broiler chickens fed 20 g of grape seed/kg diet compared to the control, as well as Gungor et al. (2021) in broiler chickens fed 5 g of grape seed/kg diet. Gungor et al. (2021) state that these results could be due to the polyphenol-rich grape seed that can affect nutrient absorption in intestinal cells.

However, grape seed supplementation tended to increase the FCR values in rabbits from the GS group than in those from the control group. This indicates that GS animals required higher quantities of their diet for each kg of body weight gain compared to the control group. Motta Ferreira et al. (1996) observed that ratio gain: food linearly decreased with the grape seed inclusion in growing rabbits.

Replacing part of the barley and maize with 10 % of grape seed led to an increase in EE level in the GS diet (25.9 vs 28.6 g/kg DM for control and GS diets, respectively), as well as an increase in DMI in the GS group, positively influencing IMF levels. The meat IMF level probably influenced the FA profile. A recent study of Martinez-Alvaro et al. (2018) showed that high-IMF resulted in greater C14:0, C16:0, C16:1, and C18:1 *cis*-9 and lower C18:0 percentages than low-IMF in rabbits. A similar trend was found in the present experimental trial, where the addition of 10 % grape seed increased C18:1 *cis*-9 level, and tended to increase also the level of C14:0, C16:0, C16:1 *cis*-9, bringing to a slight reduction of C18:0. However, it was probably the diet composition more than the IMF value that was responsible for the differences found in FA profile of meat. The tendentially higher level of total *n*-6 PUFA in the meat of animals from the GS group than in the meat from the control group was mainly due to the higher C18:2 *cis*-9 *cis*-12 concentration in GS diet, which led to a slight increase of this fatty acid in the meat of the rabbits of the GS group. These data consequently affected the *n*-6/*n*-3 ratio, which was higher in the GS group than in the control group. This difference was also influenced by the lower level of total *n*-3 PUFA in the meat of rabbits supplemented with grape seed than in the control group. The latter data may be due to the highest level of *n*-3 PUFA in the control diet. Bouzaida et al. (2021) observed similar results for *n*-6 and *n*-3 PUFA in rabbits fed a diet containing 20 % grape pomace. The data of this study show that grape seed supplementation caused an undesirable

increase of the *n*-6/*n*-3 ratio compared to the control diet. However, in our study both treatments exceeded the recommended value of 4 (Department of Health, 1996), which is considered the limit for reducing cardiovascular disease risks (McAfee et al., 2010). However, taking into account the interesting results recently obtained from Zhang et al. (2024) on growing-finishing pigs, that observed an effect of grape seed procyanidins in expression of some genes involved in lipid metabolism, the involvement of secondary metabolites could not be excluded also in the present study.

Finding systems to extend the shelf-life of meat and meat products is one of the most important challenges in order to provide products that maintain their safety and sensorial acceptability during storage to the consumer. During meat storage, lipids are generally subject to oxidation processes, chain reactions that mostly degrade FA with a high level of unsaturation (Dominguez et al., 2019), which unavoidably result in a deterioration of the organoleptic qualities of meat. During this process, primary and secondary products of lipid oxidation, such as hydroperoxides and malondialdehyde, increase rapidly. Among the variables that affect oxidative stability, the diet that the animal receives during growth plays an important role, affecting the PUFA content and the levels of antioxidant compounds in the meat, which are antagonistic to each other. In fact, whereas lipid oxidation is more intense with increasing PUFA levels (Moloney et al., 2012), the presence of antioxidants protects lipids from oxidation (Menci et al., 2023). In this contest, the high PUFA content in rabbit meat makes it highly susceptible to lipid oxidation, thus shortening its shelf-life, which can be mitigated by supplementing the diet with antioxidant compounds (Dalle Zotte & Szendro, 2011).

In this study, the addition of grape seed to the rabbit diet made the meat more resistant to lipid oxidation. In fact, according to TBARS analysis, the malondialdehyde content in the control meat increased during refrigerated storage, while it did not change in the GS group. This result was probably due to the higher content of  $\alpha$ -tocopherol in GS meat, giving it a greater antioxidant power. The antioxidant properties of  $\alpha$ -tocopherol are well known, a fat-soluble vitamin with the ability to prevent lipid peroxidation of membrane fatty acids (Ponnampalam et al., 2022). Therefore, considering the high PUFA content in rabbit meat, the addition of GS to the diet may help reduce oxidative damage during storage, thus extending the shelf-life of rabbit meat and improving the overall consumer preference for rabbit meat (Dal Bosco et al., 2012; Peiretti et al., 2012). However, the higher  $\alpha$ -tocopherol level in GS meat than in control meat was unexpected, considering the

$\alpha$ -tocopherol level of the experimental diets.

Previous studies (Viveros et al., 2011; Abu Hafsa & Ibrahim, 2018), have shown that dietary polyphenol- rich grape seed can affect nutrient absorption in intestinal cells. An in vitro study by Adámez et al. (2012) showed that grape seed contains polyphenols, such as gallic acid, catechin and proanthocyanidins, which promote the antioxidant defense system. Data from Hafsa and Hassan (2022) clearly show that the addition of grape seed can increase the antioxidant capacity of tissue-lipid and protein, attributing these results to the polyphenols present in grape seed which, by stabilizing radical intermediators, prevent oxidation. Grape seed are rich in proanthocyanidins, which scavenge free radicals and prevent oxidation (Spranger et al., 2008). In a study on growing lambs, Flores et al. (2021) showed that meat from animals whose diet was supplemented with grape pomace silage had lower TBARS values during storage than meat from the control group. Similar results were found by Jeronimo et al. (2012) integrating grape seed extract in lamb's diet. In our study, replacing 10 % of cereals with grape seed in the diet increased the total polyphenols by 30 %, compared to the control diet (5.70 vs 4.02, respectively). We hypothesized, also on the basis of data reported by other authors (Viveros et al., 2011; Abu Hafsa & Ibrahim, 2018) on the possible effect of polyphenol- rich grape seed on nutrient absorption, that GS supplementation could have had a positive effect on vitamin E absorption.

## 5. Conclusion

Adding 10 % grape seed to the diet of growing rabbits can improve the ADG and final body weight of the animals, and the data may be influenced by the higher DMI of the rabbits in the GS group rabbits compared to the control rabbits.

Grape seed supplementation had a positive effect on C18:1 cis-9 and total MUFA levels in meat. However, the ratio  $n-6$  to  $n-3$  was higher in the meat from the GS group than in the meat from the control group, consequently the lower level of  $n-3$  PUFA and the tendentially higher level of  $n-6$  PUFA in the meat of the GS group than in the meat of the control group.

Meat shelf-life was positively affected by grape seed supplementation. This result was probably due to the highest content of  $\alpha$ -tocopherol in GS meat. However, this data was unexpected, considering the comparable  $\alpha$ -tocopherol levels of experimental diets. Further research is needed to better understand this phenomenon.

## CRedit authorship contribution statement

**Manuel Scerra:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francesco Foti:** Writing – review & editing, Methodology. **Pasquale Caparra:** Writing – review & editing. **Matteo Bognanno:** Writing – review & editing. **Paolo Fortugno:** Writing – review & editing, Formal analysis. **Domenico Viglianti:** Formal analysis. **Domenico Autolitano:** Formal analysis. **Guido Mangione:** Writing – review & editing, Formal analysis. **Martino Musati:** Writing – review & editing, Formal analysis. **Luigi Chies:** Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

## Data availability

Data will be made available on request.

## References

- Abouelenen, D., Mustafa, A. M., Caprioli, G., Ricciutelli, M., Sagratini, G., & Vittori, S. (2023). Phenolic and nutritional profiles, and antioxidant activity of grape pomaces and seeds from Lacrima di Morro D'alba and Verdicchio varieties. *Food Bioscience*, 53, Article 102808.
- Adámez, J. D., Samino, E. G., Sánchez, E. V., & González-Gómez, D. (2012). In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape seeds (*Vitis vinifera* L.). *Food Control*, 24, 136–141.
- AOAC. (1995). Association of official analytical chemists. In *Official Methods Of Analysis*, Washington, DC (16th ed.). AOAC.
- Bekhit, A. E.-D. A., Hopkins, D. L., Fahri, F. T., & Ponnampalam, E. N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers, and remedies. *Comprehensive Reviews in Food Science and Food Safety*, 12(5), 565–597.
- Beres, C., Costa, G. N. S., Cabezedo, I., da Silva-James, N. K., Teles, A. S. C., Cruz, A. P. G., ... Freitas, S. P. (2017). Towards integral utilization of grape pomace from winemaking process: A review. *Waste Management*, 68, 581–594.
- Bouzaida, M. D., Resconi, V. C., Gimeno, D., Romero, J. V., Calanche, J. B., Barahona, M., & Olleta, & L. and María, G. A. (2021). Effect of dietary grape pomace on fattening rabbit performance, fatty acid composition, and shelf life of meat. *Antioxidants*, 10, 795.
- Contreras, M. D. M., Romero-García, J. M., Lopez-Linares, J. C., Romero, I., & Castro, E. (2022). Residues from grapevine and wine production as feedstock for a biorefinery. *Food and Bioprocess Technology*, 134, 56–79.
- Dal Bosco, A., Mourvaki, E., Cardinali, R., Servili, M., Sebastiani, B., Ruggeri, S., Mattioli, S., Taticchi, A., Esposito, S., & Castellini, C. (2012). Effect of dietary supplementation with olive pomaces on the performance and meat quality of growing rabbits. *Meat Science*, 92, 783–788.
- Dalle Zotte, A., & Szendro, Z. (2011). The role of rabbit meat as functional food. *Meat Science*, 88, 319–331.
- Department of Health. (1996). *Nutritional Aspects of Cardiovascular Disease. Report of the Cardiovascular Review Group Committee on Medical Aspects of Food Policy. Reports on Health and Social Subjects*. 46 pp. 1–186.
- Dhagare, L. G., Rathod, K. S., Badhe, S. R., & Patil, P. S. (2022). Effect of grape seed extract powder on shelf life of Superchilled chicken sausage. *Journal of Meat Science*, 17(1), 8–16.
- Dominguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, 8(10), 429.
- Flores, D. R. M., Patrícia da Fonseca, A. F., Schmitt, J., Tonetto, C. J., Garcia Rosado Junior, A., Hammerschmitt, R. K., Facco, D. B., Brunetto, G., & Nornberg, J. L. (2021). Lambs fed with increasing levels of grape pomace silage: Effects on meat quality. *Small Ruminant Research*, 195, Article 106234.
- Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, 226(1), 497–509.
- Frank, J., Fukagawa, N. K., Bilia, A. R., Johnson, E. J., Kwon, O., Prakash, V., Miyazawa, T., Clifford, M. N., Kay, C. D., Crozier, A., Erdman, J. W., Shao, A., & Williamson, G. (2020). Terms and nomenclature used for plant-derived components in nutrition and related research: Efforts toward harmonization. *Nutrition Reviews*, 78, 451–458.
- García, J., Nicodemus, N., Carabano, R., & De Blas, J. C. (2002). Effect of inclusion of defatted seed meal in the diet on digestion and performance of growing rabbits. *Journal of Animal Science*, 80, 162–170.
- Gray, I. K., Rumsby, M. G., & Hawke, J. C. (1967). The variations in linolenic acid and galactolipid levels in Gramineae species with age of tissue and light environment. *Phytochemistry*, 6, 107–113.
- Gungor, E., Altop, A., & Erener, G. (2021). Effect of RAW and fermented grape seed on growth performance, antioxidant capacity, and cecal microflora in broiler chickens. *Animal*, 15, Article 100194.
- Hafsa, S. H. A., & Hassan, A. A. (2022). Grape seed alleviates lindane-induced oxidative stress and improves growth performance, caecal fermentation and antioxidant capacity in growing rabbits. *Journal of Animal Physiology and Animal Nutrition*, 106, 899–909.
- Hafsa, S. H. A., & Ibrahim, S. (2018). Effect of dietary polyphenol-rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. *Journal of Animal Physiology and Animal Nutrition*, 102, 268–275.
- International Organization of Vine and Wine (OIV). (2025). State of the World Vine and Wine Sector in 2024. In *Global Data on the Wine Sector in 2024 Was Released at Its Online Press Conference*, 15 April 2025.
- IUPAC. (1987). *International Union of Pure and Applied Chemistry. Standard Methods for the Analysis of Oils, Fats and Derivatives*. Oxford: Pergamon Press.
- Jeronimo, E., Alfaia, C. M. M., Alves, S. P., Dentinho, M. T. P., Prates, J. A. M., Vasta, V., ... Bessa, R. J. B. (2012). Effect of dietary grape seed extract and Cistusladanifer I. in combination with vegetable oil supplementation on lamb meat quality. *Meat Science*, 92, 841–847.
- López-Andrés, P., Luciano, G., Vasta, V., Gibson, T. M., Scerra, M., Biondi, L., ... Mueller-Harvey, I. (2013). Antioxidant effects of ryegrass phenolics in lamb liver and plasma. *Animal*, 1–8.
- Luciano, G., Roscini, V., Mattioli, S., Ruggeri, S., Gravador, R., Natalello, A., ... Priolo, A. (2017). Vitamin E is the major contributor to the antioxidant capacity in lambs fed whole dried citrus pulp. *Animal*, 11(3), 411–417.
- Martínez-Alvaro, M., Blasco, A., & Hernández, P. (2018). Effect of selection for intramuscular fat on the fatty acid composition of rabbit meat. *Animal*, 12, 2002–2008.

- McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M. W., Bonham, M. P., & Fearon, A. M. (2010). Red meat consumption: An overview of the risks and benefits. *Meat Science*, *84*(1), 1–13.
- Menci, R., Biondi, L., Natalello, A., Lanza, M., Priolo, A., Valenti, B., Bertino, A., Scerra, M., & Luciano, G. (2023). Feeding hazelnut skin to lambs delays lipid oxidation in meat. *Meat Science*, *202*, Article 109218.
- Merli, R., Preziosi, M., & Acampora, A. (2018). How do scholars approach the circular economy? A systematic literature review. *Journal of Cleaner Production*, *178*, 703–722.
- Moloney, A. P., Kennedy, C., Noci, F., Monahan, F. J., & Kerry, J. P. (2012). Lipid and colour stability of *M. longissimus* muscle from lambs fed camelina or linseed as oils or seeds. *Meat Science*, *92*, 1–7.
- Motta Ferreira, W., Fraga, M. J., & Carabaño, R. (1996). Inclusion of grape pomace, in substitution for alfalfa hay, in diets for growing rabbits. *Animal Science Journal*, *63*, 167–174.
- Nasr, A. M., El-Din, S., El, A., Ismail, I. E., Aldhahrani, A., Soliman, M. M., Alotaibi, S. S., Bassionya, S. S., & Abd El-Hack, M. E. (2022). A comparative study among dietary supplementations of antibiotic, grape seed and chamomile oils on growth performance and carcass properties of growing rabbits. *Saudi Journal of Biological Sciences*, *29*, 2483–2488.
- Natalello, A., Khelil-Arfa, H., Luciano, G., Zoon, M., Menci, R., Scerra, M., ... Priolo, A. (2022). Effect of different levels of organic zinc supplementation on pork quality. *Meat Science*, *108731*.
- Peiretti, P. G., Gai, F., Rotolo, L., Brugiapaglia, A., & Gasco, L. (2012). Effects of tomato pomace supplementation on carcass characteristics and meat quality of fattening rabbits. *Meat Science*, *95*(2), 345–351.
- Ponnampalam, E. N., Kiani, A., Santhiravel, S., Holman, B. W. B., Lauridsen, C., & Dunshe, F. R. (2022). The importance of dietary antioxidants on oxidative stress, meat and Milk production, and their preservative aspects in farm animals: Antioxidant action, animal health, and product quality—Invited review. *Animals*, *12* (23), 3279.
- Rufino-Moya, P. J., Joy, M., Lobón, S., Bertolín, J. R., & Blanco, M. (2020). Carotenoids and liposoluble vitamins in the plasma and tissues of light lambs given different maternal feedings and fattening concentrates. *Animals*, *10*, 1813.
- Scerra, M., Foti, F., Caparra, P., Cilione, C., Rao, R., Priolo, A., Natalello, A., Luciano, G., & Chies, L. (2022). Effect of feeding pigs with bergamot by-product on fatty acid composition and oxidative stability of meat and salami. *Meat Science*, *183*, Article 108662.
- Siu, G. M., & Draper, H. H. (1978). A survey of the malonaldehyde content of retail meats and fish. *Journal of Food Science*, *43*, 1147–1149.
- Spranger, I., Sun, B., Mateus, A. M., Freitas, V., & Ricardo-Da-Silva, J. M. (2008). Chemical characterization and antioxidant activity of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chemistry*, *108*, 519–532.
- Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, *338*, 985–992.
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597.
- Viveros, A., Chamorro, S., Pizarro, M., Arjia, I., Centeno, C., & Brenes, A. (2011). Effects of dietary polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. *Poultry Science*, *90*, 566–578.
- Vizzarri, F., Palazzo, M., D'Alessandro, A. G., & Casamassima, D. (2017). Productive performance and meat quality traits in growing rabbit following the dietary supplementation of *Lippia citriodora*, *Raphanus sativus* and *Solanum lycopersicum* extracts. *Livestock Science*, *200*, 53–59.
- Zhang, Y., Zhai, Y., Wei, X., Yang, X., Deng, C., Li, O., Wang, W., & Hao, R. (2024). Effects of grape seed procyanidins on the lipid metabolism of growing-finishing pigs based on transcriptomics and metabolomics analyses. *Meat Science*, *213*, Article 109504.