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Perspectives and advantages of using olive (*Olea europaea*) by-products as a dietary supplement for rabbit production and health

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Abstract

Over the years, the olive oil market has increased considerably due to its organoleptic features and increasing awareness of the beneficial properties of olive products for human health. However, the olive oil production processes generate a variety of wastes and by-products that create serious environmental concerns because of their high phytotoxicity, but also represent an extraordinary potential source of functional compounds, such as polyphenols. This review explored the application of olive by-products as possible functional feed ingredient in rabbit nutrition. The available literature indicates that the manipulation of the rabbit diet is very reliable in producing "enriched meat" and that the bioactive fractions of olive by-products can be used to enhance meat microbial quality, fatty acid profile, and can increase the presence of compounds with natural antioxidant effect, which can exert beneficial effects on gut microbiota and animal welfare. Therefore, supplementing the diet of rabbits with olive by-products could present a sustainable option for valuable biomass, reduce the costs associated with animal feeding, and provide an "eco-green" improvement of meat quality.

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Introduction

Agro-industrial processing of food or non-food products generates a huge amount of by-products and residues that are rich in natural bioactive compounds (Kasapidou *et al*., 2015; Cappelli *et al*., 2021). The reports on global fruit and vegetable production show that residues with potential utilization after processing have been calculated in millions tons every year (Kowalska *et al*., 2017). Indeed, there is a great social, economic, and environmental concern about the effective reuse of these residues, which may have a negative impact on the environment and, on the other hand, represent a loss of valuable biomass rich in natural bioactive molecules and nutrients, which may be used for innovative food production (Santana-Meridas, 2012; Pfaltzgraff *et al*., 2013; Kasapidou *et al*., 2015; Kowalska *et al*., 2017; De Bruno *et al*., 2018). Vegetable residues constitute a valuable natural source of carbohydrates and polysaccharides; according to researchers, these by-products can present a very high content of bioactive molecules, such as proteins, vitamins, minerals, and antioxidants, such as polyphenols, flavonoids, and tannins (Grigoras *et al*., 2012; Correddu *et al*., 2020; Branciari *et al*., 2021; Vastolo *et al*., 2022). Therefore, although biomass residues produced along the food chain are not suitable for human consumption, they have considerable nutritional properties (Luciano *et al*., 2020) due to their appreciable bioactive components (Vastolo *et al*., 2022) that are useful for animal nutrition to improve animal health (Azizi *et al*., 2018; Correddu *et al*., 2020; Pinotti *et al*., 2020). As a sustainable alternative to traditional procedures, green approaches have recently been presented to recover bioactive compounds that safeguard human welfare, preserve the environment, and raise agro-

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industrial field competitiveness by a circular-green strategy. The 3R slogan “Reduce, Reuse, Recycle” should be assumed to revise the management of food waste and reduce the adverse effects on the environment of by-products (Memon *et al*., 2010; Sakai *et al*., 2011; Pinotti *et al*., 2020). Circular production models create more effective systems able to decrease both the consumption of natural resources and the amplification of wastes (Brunetti *et al*., 2022). Moreover, in the auspicious vision of the circular economy, the European Commission set the strategy “Farm to Fork Strategy – for a fair, healthy, and environmentally-friendly food system” (European Union Commission, 2020) with the intent of reducing the losses along the food chain and ensure the sustainability of food production, processing, and consumption (Vastolo *et al*., 2021).

Fruit and vegetable processing residues have historically been used in livestock feeding as main ingredients and their influence on animal performance has been extensively studied (Pfaltzgraff *et al*., 2013). Therefore, studies on the application of these by-products have attracted great interest, not only from an environmental point of view, but also from a human health perspective in producing functional foods that contain natural extracts (Cappelli *et al*., 2021).

The olive (*Olea europea*) cultivation and the olive oil industries play an important economic and social role in the Mediterranean basin (International Olive Council, 2017; Tufarelli *et al*., 2022a). Apart from the Mediterranean area, olive tree cultivation is also increasing in USA, Middle Eastern, and African countries (Selim *et al*., 2022). In recent years, the olive oil market has increased substantially due to organoleptic characteristics and rising awareness of the health benefits of olive products. However, cultivating olive trees and producing industrial and table olive oil generates enormous volumes of solid waste and dark liquid effluents, such as olive pomace, leaves, and olive oil mill wastewaters. Thus, these by-products cause an economic problem for producers and pose serious environmental concerns (Salomone *et al*., 2012).

Therefore, examining all the potential circular economic paths for the reuse of compounds from the olive oil supply chain is crucial (Stempfle *et al*., 2021). Olive by-products are rich in bioactive principles; indeed, they deliver a considerable amount of valuable organic acids, carbohydrates, proteins, fibre, and phenolic materials, which are characterized by a high variability in chemical composition between the various wastes and depend on the olive oil extraction method. Furthermore, innovative food applications and enhanced technological functions are now available to transfer these high value products in food directly or through the animal diets (Pinotti *et al*., 2020). Indeed, researchers have focused their interest on the production of new technologies for extracting and recovering bioactive chemicals from olive by-products and using them as functional feed ingredients in animal nutrition (Brunetti *et al*., 2022). Thus, the use of olive by-products as a part of animal diets can be a very effective strategy for recovering these healthy molecules and improving the functional value of meat and meat production.

Thus, using by-products in animal feed may ameliorate the technological quality of the final product and, on the other hand, constitute an economically and environmentally-friendly option in the livestock arena, raising productivity and sustainability (Berbel *et al*., 2018; Foti *et al*., 2021). Moreover, in recent decades, the European ban of the use of synthetic additives and antibiotic growth promoters (AGPs) in animal feed has emphasized the necessity to research alternative substances and additives that promote animal health, benefit growth performance, and improve meat quality (Bosetti *et al*., 2020; De Cara *et al*., 2023). This also considers the growing interest of the consumer on the link between diet and health, which has increased the demand for animal-derived functional foods for human consumption and a pressing requirement for ‘clean label’ foods that are safe and health-promoting.

Recent studies indicate that the use of natural feed additives (such as prebiotics, probiotics, synbiotics, organic acids, essential oils, antibodies, enzymes) affect the growth, function, and health of living organisms and they have been recognized as antimicrobials, antioxidants, antioxygenic, and antiparasitic (Madhupriya *et al*., 2018; Morshedy *et al*., 2021; Brunetti *et al*., 2022; Vastolo *et al*., 2022; Wickramasuriya *et al*., 2022). Moreover, when they are used as additives in animal diets, they can also act as growth promoters since they don’t negatively affect animal health or the quality of animal products for human consumption (Falcao-e-Cunha *et al*., 2007; Akyildiz *et al*., 2016: Dalle Zotte *et al*., 2016; Brunetti *et al*., 2022). Owing to the rich rate of high value-added compounds, olive by-products can be employed to enrich other food products directly or indirectly, present lower toxicity, and are free of undesirable residues, compared to inorganic chemicals or antibiotics. Consequently, the bioactive molecules found in olive by-products, in particular, the fatty acids and polyphenols, can be used to make nutritional supplements and can represent an alternative natural dietary strategy based on plant-derived metabolites instead of synthetic products (Correddu *et al*., 2020; Oluwafemi *et al*., 2020).

Of the livestock products, rabbit meat is well appreciated for its high nutritional and dietetic properties that meet the favour of consumers. This animal-derived product is considered very healthy because it is lean, possesses a relatively high content of unsaturated fatty acids (60% of total FA), is rich in proteins (20–21%), and the amino acids are of high biological value; furthermore, it is low in cholesterol and sodium and rich in potassium, phosphorus, and magnesium (Bielanski *et al*., 2000; Dalle Zotte, 2002). Thus, rabbit meat could be a precious food in human nutrition, also considering that dietary fortification has been seen to be very effective in increasing the provision of the main nutrients. However, due to its significant concentrations of PUFA, rabbit meat presents a high susceptibility to lipid oxidation during storage, manifested by adverse changes in flavour, colour, texture, and nutritive value, with the possible production of toxic compounds (Trebusak *et al*., 2014).

Over the years, numerous studies have been conducted to determine how rabbit meat can be nutritionally enhanced and its shelf life extended to provide health-promoting effects for consumers. Studies involving humans and animals (*in vivo* and *in vitro*) have evidenced that dietary supplementation with olive oil and olive by-products has potentially advantageous biological effects resulting from their antioxidant, antimicrobial, and anti-inflammatory activities. Indeed, due to the high ratio of bioactive components, olive by-products represent a natural raw material for the recovery of valuable nutrients, such as polyphenols or aromatic oil; thus, their inclusion in rabbit diets could be a convenient strategy to increase the intrinsic quality and health conditions of the rabbits.

Based on these considerations, this review aimed to provide an overview of the recent knowledge on the application of olive by-products as a functional feed ingredient in the nutrition of growing rabbits for meat production. A brief description of the most represented bioactive molecules in the different olive by-products is also reported.

Rabbit meat: Main properties and perspectives for its improvement

The demand for functional foods has grown in recent years, also due to the increasing consumer knowledge of the link between health and diet. Consumers demand the production of “clean, natural, and eco-green”-label food products and pay attention on the major determinants of food quality such as sensory characteristics, shelf life, nutritional value, and health enhancers (Wenk, 2000; Kasapidou *et al*., 2015). Meat and meat products may be reputed as functional foods to the extent that they contain numerous compounds thought to be functional (Dalle Zotte *et al*., 2011).

Rabbit meat, per se, is characterized by a valuable nutritional composition and is thus appropriate for the modern consumer. This aspect represents the reason for promoting its consumption by nutritionists. Moreover, since the rabbit is a monogastric animal, dietary changes and/or supplementation with health-promoting ingredients are efficient strategies to further increase the nutritional quality of its meat (Dalle Zotte *et al*., 2011).

The rabbit is a highly specialized herbivore, being a monogastric hindgut fermenter. Due to its digestive physiology, rabbits effectively turn the proteins contained in cellulose-rich plants into food containing high-value animal proteins (Dalle Zotte, 2014; Cappelli *et al*., 2021). In fact, rabbit meat is rich in protein with a high essential amino acid content and it has a low fat content with an excellent proportion of saturated, monounsaturated, and polyunsaturated fatty acids. Rabbit meat also has a low cholesterol and sodium content. In addition, it is a good source of potassium, phosphorus, selenium, and B vitamins and is one of the richest sources of vitamin B12 (Cullere *et al*., 2018).

Moreover, manipulating rabbit diet is very effective in producing enriched meat and some bioactive compounds can be easily incorporated into the meat through the diet to obtain products considered functional (Dalle Zotte *et al*., 2011). One of the main aims of meat researchers for the nutritional improvement of meat is to reduce the saturated FA and increase the unsaturated FA in fat deposits. However, when the dietary manipulation enhances the content of unsaturated FA, this tends to increase the oxidative susceptibility of muscle tissue. Many investigations have shown that increasing the content of n-3 PUFA in diet has a positive effect on meat fatty acid composition (Dal Bosco *et al*., 2012; Trebusak *et al*., 2014; Branciari *et al*, 2021), but in return, this makes muscle tissue more susceptible to lipid oxidation (Bielanski *et al*., 2008; Zsédely *et al*., 2008; Trebusak *et al*, 2014). Thus, to prevent the oxidation, the addition of natural antioxidants in the rabbit diet seems to be an effective strategy to improve meat stability.

Therefore, appropriate dietary supplementation that is balanced in fat sources and antioxidant intake is fundamental to improving nutritional value and meat-product stability, shelf-life, and sensorial properties of rabbit meat. The dietary incorporation of olive by-products would satisfy both the requirements and the demands of the consumer since the biocompounds present in them can increase both the content of unsaturated fatty acids and the content of substances with antioxidant activity.

Use of olive by-products in animal production: Main goals and advantages

In recent years, researchers have focused their attention on applying olive by-products and/or their bioactive compounds to ameliorate the nutritional profile of food products, find novel natural additives, and reduce costs related to waste management and animal feeding.

Many generations attest to the fact that olive oil is an essential component of the healthy Mediterranean diet and represents the primary fat source in the MedDiet (Frankel *et al*., 2013; Pappas *et al*., 2019). In this context, olive oil consumption, in particular extra virgin olive oil (EVOO), plays an essential role as the main source of natural bioactive compounds, which exert the well-known beneficial health effects of the diet (European Union Reg EU 432/2012; Gaforio *et al*., 2019; Sarapis *et al*., 2022).

EVOO's ability to improve health conditions is due to several components, including its high content of monounsaturated fatty acids (MUFAs), in particular oleic acid, which can improve α-linolenic acid (ALA) conversion into longer-chain n-3 polyunsaturated fatty acids (PUFAs), leading to greater health benefits in terms of cardiovascular disease (CDV) (Wahle *et al*., 2004), metabolic diseases, inflammatory and autoimmune diseases, as well as the prevention of breast and colon cancer (Alarcon de la Lastra *et al*., 2001). The Food and Drug Administration (FDA) had recognized olive oil (23 g/day) as a qualified health claim to decrease the risk of coronary heart disease (FDA, 2004). To deliver benefits for human health, researchers recommend the absolute dietary intake of long chain n-3 PUFA and a decrease in the n-6/n-3 ratio to 5 as a maximum threshold value (Dalle Zotte *et al*., 2011; Cullere *et al*., 2018).

Numerous studies reported that olive oil health effects might also attributed to microconstituents, such as phenolic compounds or phenolic glycosides, such as oleuropein, hydroxityrosol, and tyrosol. In fact, their relevance has been recognized by the European Food Safety Authority (EFSA, 2011) with a 'health claim' related to specific EVOO phenolic compounds. According to the scientific opinion of EFSA (2011), a daily amount of 5 mg of hydroxytyrosol (3,4 dihydroxyphenylethanol; 3,4-DHPEA or HT) and its derivatives is responsible for the explicated health claim "protection of blood lipids from oxidative stress" (EFSA 2011). In an experimental study on humans, Colica *et al*. (2017) detected that the regular intake of 15 mg/kg HTyr resulted in the modification of body composition parameters and modulated the antioxidant profile and the expression of inflammation and oxidative stress-related genes in atherosclerosis (Finicelli *et al*., 2022). Moreover, the phenolic fraction of EVOO, except for oleic acid, also acts as promoting factor in the growth or survival of beneficial gut bacteria (mainly *Lactobacillus* strains) and inhibits the proliferation of some pathogenic bacteria (Martìn-Pelàez *et al*., 2017; Luisi *et al*., 2019). The available literature consistently emphasises that the diet influences the intestinal ecosystem and the functional capacity of the gut microbiota, which is recognized as a key factor in driving metabolic activity and is involved in the regulation of host immunity (Delzenne *et al*., 2011; Danneskiold-Samsøe *et al*., 2019).

Since the highest content (up to 70%) of these bioactive molecules is found in the unwrapped part and in the outer parts of the olive fruit, researchers have focused their attention on the use in animal feeding of olive processing by-products such as **olive pomace**, **olive cake**, **olive mill waste waters**, and **olive leaves, the** main characteristic and biocompounds of which are briefly described below (Contreras-Calderòn *et al*., 2011; Nunes *et al*., 2016; Sagar *et al*., 2018; Romeo *et al*., 2021; Foti *et al.*, 2022).

Olive pomace (**OP**) is a heterogeneous biomass of semi-solid consistency remaining after olive oil extraction, with a considerable moisture and oil content that depends on the cultivation region, the ripening period, and the extraction system utilized (Meziane, 2011; Akay *et al*., 2015). Its major ingredients are sugars, proteins, fatty acids (oleic acid and other C2–C7 fatty acids), polyalcohols, polyphenols (Rodriguez *et al*., 2008; Mirabella *et al*., 2014), which can be gained using different extraction methods. OP chemical compounds, especially phenols, stand out as a promising valuable by-product. Several phenolic compounds have been detected: oleuropein, hydroxytyrosol and tyrosol derivatives, iridoid precursors, secoiridoids and derivates, flavonoids, lignans, and phenolic acids. In particular, the most abundant phenolic compound in olive fruit, namely oleuropein, has been found at high concentrations in OP (up to 0.9%) (Savournin, 2001; Sanchez de Medina *et al*., 2012).

Olive cake is constituted by the olive skin, crushed pulp, and kernel shell that come after oil extraction; it still contains some oil (9%) and ~25% water. The olive cake is also called "fatty olive cake" if the cake is not subjected to solvent extraction for oil separation. Generally, in animal feed, the olive cake is mixed with molasses (considering its lower palatability) and is utilized as a substitute for fibre because of its high cellulose content (Ferrer *et al*., 2021). The olive cake is characterized by a low crude protein content, a high crude fibre content, and up to 15% ether extract which is composed, principally, of monounsaturated fatty acids (mainly C18:1 cis-9 or oleic acid) (Molina-Alcaide *et al*., 2008). The use of olive cake in animal feeding constitutes a sustainable option to the disposal of biodegradable organic matter, decreasing dietary costs and permitting the rational utilization of this residual biomass. Furthermore, olive cake is a considerable source of phenolic and flavonoid compounds, so it has a wide range of biological properties. In accordance with several reports, olive cake contains phenolic compounds such as oleuropein, caffeic acid and hydroxytyrosol catechol (Selim *et al*., 2022). Allouche *et al*. (2004) identified tyrosol, rutin, vanillic acid, p-coumaric acid, verbascoside, and oleanolic acid.

Olive mill waste water (**OMWW**) is a liquid effluent derived mainly from the water used for the various stages of oil production and vegetable water from the fruit, and amounts to 0.5–3.25 m^3 per 1000 kg of olives (Paraskeva *et al*., 2006; Kapellakis *et al*., 2012; Gerasopoulos *et al*., 2015b). The physicochemical features of OMWW are highly influenced by the conditions of soil and climate, cultivar, ripeness state and, above all, by oil extraction method. The OMWW is black and has a typical and intense aroma (Foti *et al*., 2021).

OMWW consists of 90% water, a minimal amount of organic compounds and mineral salts, and contains tannins, lignin, long chain fatty acids, reduced sugars, proteins, and phenolic compounds, which are toxic to microorganisms and plants (Paixao *et al*., 2002; Paraskeva *et al*., 2006). However, despite the toxicological effects on the environment, these compounds may have nutritional relevance in human diets owing the several health benefits, such as the inhibition of low-density lipoprotein oxidation, platelet aggregation, free radical reduction, production of leukotriene for human neutrophils, and *in vitro* antimicrobial activity (De Marco *et al*., 2007; Nunes *et al*., 2016). The OMWWs contain a substantial amount of bioactive compounds, namely phenols. Most of the phenolic portion present in olives is found in OMWW (>53%) and OP (45%), with only 2% of the initial content remaining in virgin olive oil (Rodis *et al*., 2002; Di Nunzio *et al*., 2019). The OMWW is a rich source of phenols, as it comprises 98 g/100 g of the total phenolic content of olive fruit and can therefore be considered to be of great potential (De Bruno *et al*., 2018).

The phenolic compounds present in OMWW are hydroxytyrosol, tyrosol, verbascoside, acids (such as caffeic, gallic, vanillic, and syringic), and polymeric substances (Obied *et al*., 2008a; Frankel *et al*., 2013; D'Antuono *et al*., 2014). Therefore, OMWW could be exploited as a possible cheap, starting matrix for the extraction of antioxidants in several fields, not least the food industry, where they could be used for fortifying and prolonging the shelf life of final products (De Marco *et al*., 2007; Obied *et al*., 2008b; Foti *et al*., 2021).

Olive leaves (**OL**) have been used for different purposes as food preservatives, additives in many products, cosmetics, and human health (Roselló-Soto *et al*., 2015). Olive leaves are mainly used to obtain olive leaf extract (OLE), tea, powder, and capsules (Ghanbari *et al*., 2012; Selim *et al*., 2022). Oleuropein is the most abundant phenolic compound in OL, followed by hydroxytyrosol (Benavente-Garcia *et al*., 2000; Dub *et al*., 2013; Nunes *et al*., 2016). Cavalheiro *et al*. (2015) determined OL fatty acids and showed that unlike olive oil, where the major unsaturated FA present is oleic acid, the predominant fatty acid in the OL lipid fraction was linolenic acid. Considering that linolenic acid, after metabolic pathways, results in long-chain PUFA of the n-3 series, OL can be considered a source of long-chain PUFA.

Several investigations have outlined the antioxidant, anti-inflammatory, immunomodulatory, analgesic, antimicrobial, antihypertensive, anticancer, and anti-hyperglycaemic activities of olive oil by-products (Foti *et al*., 2021). In recent decades, the valorization of these by-products in the food-chain represents a new challenge for olive mills and responds to the strong demand for innovation in food with a view to the creation of virtuous recycling (Foti *et al*., 2022). With this in mind, olive oil by-products can be used as feed supplements to improve animal reproductive and productive performance, health status, and welfare in order to obtain animal-derived functional foods containing natural extracts from the olive plant.

According to Ibrhaim *et al*. (2021) and Papadomichelakis *et al*. (2019), including olive oil by-products in the feed of growing animals has a positive impact for the following reasons. Firstly, olive by-products are considered as a low-cost complementary energy source due to their high oil content (Al-Harti, 2017). Secondly, they have more PUFAs, which account for meat fatty acid composition (Molina-Alcaide *et al*., 2008). Thirdly, their polyphenol content can be considered as an excellent source of natural antioxidants (King *et al*., 2014; Gerasopoulos *et al*., 2015a,b; Tufarelli *et al*., 2016), which help in delaying oxidative consequences in muscle tissues.

Thus, olive by-products may be considered a feed additive as per EFSA (2008) definition, because of the "favourable effects":

1- the sensory characteristics and acceptance of products (i.e., antioxidants and colorants);

2- the nutritional value of products (i.e., long chain PUFA, conjugated linoleic acid);
3- the microbial quality of products.

Effects of olive by-products on meat quality, oxidative status, and microbial spoilage

The advantage of using olive byproducts as feed supplements to improve performance, reduce oxidative stress, and fortify meat antioxidant status has been demonstrated in different production animals, such as lambs and goats (Luciano *et al*., 2013; Hukerdi *et al*., 2019), chickens (Gerasopoulos *et al*., 2015b; Tufarelli *et al*., 2016; De Cara *et al*., 2023), rabbits (Dal Bosco *et al*., 2012; Branciari *et al*., 2021), beef cattle (Branciari *et al*., 2015, Chiofalo *et al*., 2020), and pigs (Joven *et al*., 2014; Tsala *et al*., 2020). Furthermore, several studies have shown that dietary supplementation is effective also for the improvement of nutritional quality and quantity of animal products like milk, cheese (Abbeddou *et al*., 2011; Terramoccia *et al*., 2013; Vargas-Bello-Perez *et al*., 2013; Branciari *et al*., 2014; Roila *et al*., 2019; Chiofalo B. *et al.*, 2020), and eggs (Abd El-Samee *et al*., 2011; Dedousi *et al*., 2022).

According to the available literature, dietary supplementation with by-products from olive oil processing might enrich the incorporation of bioactive compounds in rabbit muscle, influencing not only the microflora and the oxidative stability of meat, but also the nutritional profile of the products (Simitzis *et al*., 2018). In particular, the FA composition of olive by-products may have an advantageous influence on the intramuscular FA level (Molina-Alcaide *et al*., 2008). The olive by-products in rabbit diets influence meat quality, such as intramuscular fat and unsaturated FA contents. Indeed, the intramuscular FA profile of rabbit meat has an important role as MUFA, including oleic acid (18:1 n-9), have been shown to reduce plasma total cholesterol and low-density lipoprotein, and their consumption is highly recommended to prevent cardiovascular diseases (Gurr *et al*., 1989).

Many studies report that the inclusion of olive by-products in the diet of farm animals increases MUFA levels (especially oleic acid) in the meat of rabbits (Dal Bosco *et al*., 2012), broilers (Papadomichelakis *et al*., 2019), pigs (Tsala *et al*., 2020), lambs (Luciano *et al*., 2013), ewe milk (Vargas-Bello-Perez *et al*., 2013), and egg yolks (Dedousi *et al*., 2022). Dal Bosco *et al*. (2012) demonstrated that the content of intramuscular oleic acid and MUFA of rabbits fed different olive pomaces (OP) were proportional to oleic acid content of the by-product. The Authors also reported that dietary treatment with OP resulted in a modification of the fatty acid profile of meat with a marked increase in MUFA, and they concluded that feeding rabbits with OPs could enrich meat in precious FA that had beneficial effects on human health. Furthermore, rabbits fed supplemented diets had the greatest thiobarbituric acid reactive substances (TBARS) content and were therefore more susceptible to lipid peroxidation. Mattioli *et al*. (2018) reported that rabbits fed diets enriched with 10% olive leaves (OL) and 10% OL fortified with selenium (SeOL) showed a high amount of oleic acid and a positive trend for MUFA compared to the control group. Moreover, Papadomichelakis *et al*. (2019) found that in broiler chickens, dietary OP increased intramuscular oleic acid and MUFA in proportion to the inclusion in the finisher diet, as observed in prior studies (Sanz *et al*., 2000; Zhang *et al*., 2013a). In Japanese quail reared at different stocking densities, adding an OL extract with an oleuropein level of up to 400 ppm decreased the proportion of total SFA and increased total PUFA and n-3 and n-6 FA. Consequently, dietary oleuropein supplementation improved the quality of breast muscle lipids by lowering SFA proportions and enhancing PUFA content (Bahsi *et al*., 2016).

In finishing pigs, the increasing inclusion of olive cake linearly increased the oleic acid and MUFA levels in adipose, while meat SFA contents were decreased (Joven *et al*., 2014). An increase of MUFA concentration in the meat of pigs fed partially-defatted olive cake-supplemented diets (120 g/kg) was also observed without other side effects on growth performance, carcass quality, and microbial counts (Ferrer *et al*., 2020). Similarly, Chiofalo *et al*. (2020) found that the addition of between 7.5 and 15% OP influenced the performance of beef cattle by improving meat tenderness and modifying the meat quality index, including intramuscular fat and UFA.

Dietary olive cake supplementation of up to 5% in dairy cows increased UFA (mostly oleic acid, vaccenic acid, and CLA) and decreased SFA (short- and medium-chain FA) in derived cheese, suggesting a positive role of olive cake in increasing the nutritional and nutraceutical properties of the cheese (Chiofalo *et al*., 2020). Recently, Dedousi *et al*. (2022) reported that dietary supplementation with dried olive pulp reduced the content of total SFA, increased the percentage of PUFA, and improved the PUFA to SFA ratio in eggs.

Many trials have shown that enriching the content of n-3 PUFA has positive effect on FA content, but has a negative impact on lipid oxidation in rabbit meat, which might be improved by the implementation of antioxidants (Bielanski *et al*., 2008; Zsédely *et al*., 2008; Trebusak *et al*., 2014). Furthermore, many Authors (Rey *et al*., 2020; Xie *et al*., 2022) have focused on replacing synthetic additives with natural antioxidants; olive polyphenolic compounds seem to be an effective alternative to improving meat oxidative stability and microbial quality (Branciari *et al*., 2021; Foti *et al*., 2021). Studies both *in vivo* and *in vitro* were carried out to assess the antioxidant capacity of olive polyphenols to fortify meat and meat products through the addition of exogenous products after slaughter. Furthermore, these compounds have been also used for producing packaging materials (Bermúdez-Oria *et al*., 2018; Da Rosa *et al*., 2019; Khwaldia *et al*., 2022).

Through their antioxidant activity, olive by-products can provide multidimensional improvement in stored meat products, including colour retention, retardation of microbial growth, retardation of fat deterioration, and, ultimately, extended shelf-life (Foti *et al*., 2022). Galanakis *et al*. (2018) collected data related to the addition of an olive oil by-product (olive mill waste waters, OMWW) extract to fortify meat and meat products. Antioxidant supplementation improved hygienic conditions and rheological features of the final products. Hayes *et al*. (2009) tested aqueous phenolic extracts (100–300 mg/l) from olive leaves as well as lutein (100–300 mg/l), sesamol (500–2000 mg/l), and ellagic acid (300–900 mg/l) against oxymyoglobin oxidation and lipid oxidation in bovine and porcine muscle model systems. The lipid oxidation decreased following the addition of each of these natural antioxidants, suggesting that their application in meat products enhanced shelf-life characteristics (Gerasopoulos *et al*., 2015b; Galanakis *et al*., 2018). According to Foti *et al*. (2021), adding such compounds not only inhibits the growth of pathogens, reducing spoilage microorganisms in meat during storage, but also extends the shelf life through their antioxidant potential.

In particular, phenolic fractions of by-products have been shown to inhibit or delay the rate of growth of a vast range of gram-positive and gram-negative bacteria (Fasolato *et al*., 2015; Nazzaro *et al.*, 2019; Branciari *et al.*, 2021). *In vivo* assays showed that phenolic compounds from trees and leaves presented a potent antioxidant activity; the addition of only 5 g *O. europaea* leaves/kg in turkey diets markedly increased the oxidative stability of breast fillets during refrigerated storage and was equal to 150 mg/kg α-tocopherol acetate supplementation (Botsoglou *et al*., 2010). The inclusion of 6% linseed oil and 1% *Ganoderma lucidum* (Reishi mushrooms) or *O. europaea* leaves in rabbit diets both enhanced MUFA content and balanced the oxidative status of the treated meat. The supplemented group tended to have an increase in meat oxidative stability, as detected by modestly decreased MDA values in all stored and heat-treated conditions. The activity of *O. europaea* leaves was more efficient in cooked samples (Trebusak *et al*., 2014).

Recently, Branciari *et al*. (2021) demonstrated that rabbit dietary fortification with low (150 mg/kg) and high doses (280 mg/kg) of an extract of polyphenols derived from OMWW could influence the growth of meat microflora by enriching the level of these bioactive compounds in the muscle. The analytic determination of polyphenol content was conducted in both feed and meat (*Longissimus lumborum* muscle, LL) samples. The inclusion of OMWW in diets clearly influenced the total biophenol content in feed and muscle tissue. In the tested feeds, hydroxityrosol and verbascoside were the prevalent chemicals, followed by tyrosol and pinoresinol. As expected, the phenol metabolite contents were higher in the muscle of the treated than the control group. In particular, the hydroxityrosol sulphates (HT-3-S and HT-4-S) were ~three times higher in the muscle of animals fed with OMWW supplement at low concentration (0.60–1.25 mg/kg) than in rabbits that were fed the control diet (0.16–0.40 mg/kg). Furthermore, the content of these compounds was ~two-fold higher in rabbits fed with the highest concentration in the diet (1.11–2.52 mg/kg).

The microbiological determination was conducted on the cranial part of the LL at time 0 and after 3, 6, 9, and 12 days of storage. The microbial evolution of refrigerated LL muscle was carried out using the total viable count (TVC), psychrotrophic counts (TPC), lactic acid bacteria (LAB), and counts of *Pseudomonas* spp. and Enterobacteriaceae. The antimicrobial activity of phenolic compounds varied among the microbial populations and phenolic integration levels. The feeding strategy reduced *Pseudomonas* spp. growth starting from 6 d of storage in the group fed the highest concentration, but did not interfere with the growth of TVC, TVP, LAB, and Enterobacteriaceae. This is in contrast with previous studies that have referred to antimicrobial activity on TVC count, LAB count, and Enterobacteriaceae exerted by the addition of polyphenolic compounds through dietary supplementation with liquorice extract (Dal Bosco *et al*., 2019), oregano essential oils (Soultos *et al*., 2009), and onion and cranberry extracts (Konè *et al*., 2019) in rabbit diets.

Dal Bosco *et al*. (2012) evaluated the effects of a dietary supplementation by adding 5% of three types of olive pomace (OP) and a mixture of them derived from different olive cultivars (Coratina, Maraiolo, Frantoio, and Ogliarola), characterized by different phenolic contents. The three diets supplemented with 5% OPs yielded differences in the performance and meat quality of growing rabbits. The data demonstrated that meat quality could be seriously affected by the quality of the OP used in the rabbit diets and that the meat oxidation values tended to decrease in the OP-supplemented groups, compared to the control group. The rabbits fed the diet containing the cultivar with the highest phenolic concentration had the highest oxidative stability and nutritional value, as revealed by the low concentration of lipid peroxidation and the high nutritional indices, respectively. The lipid peroxidation level in the muscle, measured by the value of the absorbance of TBARs, revealed that, in the meat of OP-treated rabbits, the extent of peroxidation was less than in the control group. This suggests that the high polyphenol content of the supplemented diet prevented the oxidation of unsaturated lipids, contributing to the preservation of meat and resulting in a high nutritional index of meat from the treated rabbits. Furthermore, Dal Bosco *et al*. (2012) observed that rabbit meat from the group fed the diet supplemented with OP from the Coratina cultivar (50 g/kg), despite its relatively high hydroxytyrosol content, presented reduced oxidative stability and was more susceptible to lipid peroxidation. The Authors concluded that only olive by-products of high quality (in terms of pro-oxidant/antioxidant balance and high polyphenol content) could ensure an advantageous meat oxidative stability.

Oxidative stress also causes direct damage to proteins or leads to chemical modification of amino acids (Andreadou *et al.*, 2006). Recent studies have shown that protein oxidation can induce protein polymerization and aggregation, affecting their digestibility and reducing the nutritional value of the meat (Zhang *et al*., 2013b; Gerasopoulos, 2015a). According to Tufarelli *et al*. (2022a), this reaction seems to be directly influenced by the level of lipid oxidation in meat. Moreover, Gerasopoulos *et al*. (2015a) reported that there was evidence that oxidation products from protein and lipids could further increase the oxidation (Faustamn *et al*., 2010). Protein oxidation increases the protein content in carbonyls, which then serve as biomarkers of general oxidative stress (Dalle-Donne *et al*., 2003). It was found by Gerasopoulos *et al*. (2015a) that incorporating 4% OMWW into broiler feed markedly decreased the protein carbonyl content in meat; TBARS content was also substantially decreased in groups that received OMWW by 50.70 and 13.60%, respectively, in muscle; by 33.5 and 11.8%, respectively, in heart; and by 23.0 and 17.9%, respectively, in the liver, compared to the control group.

Recently, De Cara *et al*. (2023) conducted a study to demonstrate a linear and negative correlation between plasma antioxidant biomarkers and meat lipid oxidation by including an olive leaf and grape-based by-product (2g/kg OLG-mix) in broiler diets. Meat from birds supplemented with the OLG-mix resulted in a tendency to have lower TBARS production compared to groups without supplementation; plasma SOD concentrations of treated groups showed a marked increase, confirming that the supplemented diet fortified oxidative status and meat stability.

Preservation of gut microbiota and antimicrobial activity for improving rabbit health and performance

The control of oxidative stress is relevant in achieving adequate growth and a good state of health (Forman *et al*., 2021). Under physiological conditions, all cells produce reactive oxygen species (ROS), which can cause cellular damage if not captured in time by the body's antioxidant systems (Evans *et al*., 1997). Therefore, an imbalance in the organism's antioxidant and oxidant capacity can lead to diseases and physiological alterations.

The diet can influence the oxidative processes in the body and may lead to biological damage, health disorders, lower growth rates, and economic losses (Tufarelli *et al*., 2016). Supplementing animal diets with antioxidants improves the plasma's redox status balance and prevents oxidative damage by protecting the body from free radicals (Puvaca *et al*., 2018; Tufarelli *et al*., 2023).

The fruit of the olive tree (Kalogeropoulos *et al*., 2014) and its by-products (Botsoglou *et al*., 2013; King *et al*., 2014; Gerasopoulos *et al*., 2015a,b) contain several antioxidants, namely phenols, which can potentially scavenge free radicals and provide antioxidant protection. These positive effects act on both plasma and meat oxidative status. Moreover, researchers report that the antioxidant action of these compounds can have beneficial effects on the gut microbiota.

The first requirement for a dietary compound to be an *in vivo* antioxidant in an organism is that it enters the blood circulation. Animal and human studies show that olive oil by-products are well absorbed. Many reports (Vissers *et al*., 2002; Tan *et al*., 2003) have shown that oleuropein is rapidly absorbed after oral administration, with maximum plasma concentration occurring 2 h after administration (Andreadou *et al*., 2006). Polyphenols are directly absorbed or metabolized in the intestine or transformed into active metabolites, where they might exert a local action in relation to their interaction with gut microbiota (Luisi *et al*., 2019). Branciari *et al*. (2021) demonstrated that olive-derived phenolic supplementation in rabbit diets produced mainly polyphenol sulphate metabolites, confirming that these molecules were absorbed in gut (Corona *et al*., 2009; Rubiò *et al*., 2014; Branciari 2021). Tufarelli *et al*. (2016) reported that the absorption of hydroxytyrosol, the most important metabolite of oleuropein, takes place in the small intestine and colon with an absorption rate that varies according to the animal species (Visioli *et al*., 2002). It has been reported that polyphenols in some environments may exert pro-oxidant effects or interact with the gut microbiota, where they potentially modulate the oxidative status of the intestinal barrier, inflammation, and immune response of the host (Lipinski *et al.*, 2017; Deiana *et al.*, 2018; Luisi *et al.*, 2019; Abd El-Moneim *et al.*, 2020; De Cara *et al.*, 2023).

The addition of olive phenols to mixed feed of many livestock species may enhance the protective mechanism of gut microbiota against bacterial disease, promote digestion and absorption of nutrients and, as a result, improve performance (Sayehban *et al.*, 2015; Tufarelli *et al.*, 2016; Berbel *et al.*, 2018; Mancini *et al.*, 2018; Tzamaloukas *et al.*, 2021; Tufarelli *et al.*, 2022b). Tufarelli *et al.* (2016) reported a marked improvement in the growth performances in broilers fed extra-virgin olive oil over other dietary fat sources. In the same study, the intestinal histological features indicated that olive phenol supplementation increased the intestinal villus height and crypt depth, raising the absorption of nutrients and improving the performance score. The Authors postulated that the favourable results of the EVOO diet on growth performance of chickens could be explained by the positive impact of this oil on the reduced passage rate of the digesta through the gastrointestinal tract, facilitating better nutrient absorption and utilization (Poorghasemi *et al.*, 2013, 2015; Tufarelli *et al.*, 2016).

In a study conducted by Sayehban *et al.* (2016) the inclusion of olive pulp (OP) (100 g/kg) in broiler diets increased absolute and relative jejunum weights and length, resulting in an increase in lipid absorption and serum levels in comparison to other experimental groups. The jejunum is the main site of nutrient absorption in the gastrointestinal tract, and increasing its size is highly desirable and correlated with greater absorption of nutrients (Macari, 2008). Furthermore, considering that the gastrointestinal tract is the largest organ of the body's immune defence in animals, the presence of antioxidants and antimicrobial substances in this environment may influence immune organ size and weight, thus resulting in less inflammatory and chemotactic local reaction (Sayehban *et al.*, 2016). Lastly, the antioxidant compounds may enhance the antioxidant enzyme activities, reducing the intensity of lipid peroxidation and the generation of free radicals (Sreelatha *et al.*, 2009), thus, preventing morphological changes and oxidative damage of intestinal microflora. Al-Sagheer *et al.* (2017) examined the influence of supplemental dietary extra virgin olive oil (EVOO), gallic acid (GA), or lemongrass essential oil (LGEO) on growth performance, nutrient digestibility, carcass traits, lipid peroxidation, haematological, and antioxidative status in growing rabbits under heat stress conditions. In growing rabbits, studies confirm that heat-stress is the most remarkable cause of oxidative stress linked with a drastic formation of free radicals and ROS.

Oxidative stress has a detrimental effect on feed intake, body weight gain, feed efficiency, reproductive performance, and health of rabbits (Marai *et al*, 2002; Finzi *et al.*, 2010; Al Sagheer *et al.*, 2017). High environmental temperature decreases growth indices, probably due by the amplification ROS, which oxidize and destroy cellular structures and impair intestinal tissues (Al-Sagheer *et al.*, 2017). Additionally, heat stress has been reported to suppress diverse components of the immune system, enhancing vulnerability to various pathologies (Aggarwal *et al.*, 2013). Thus, antioxidant feed supplementation has been recommended for protecting and mitigating the impact of stress on health and productivity (Lykkesfeldt *et al.*, 2007; Abuelo *et al.*, 2015; Gerasopoulos *et al.*, 2015a; Al Sagheer *et al.*, 2017). In their study, Al-Sagheer *et al.* (2017) reported that dietary supplementation of EVOO, GA, or LGEO enhance growth performance, nutrient digestibility, lipid peroxidation, and antioxidative status of growing rabbits. In fact, rabbits supplemented with EVOO, GA, or LGEO showed substantially higher values of weight gain (14.57, 11.29, and 14.90%, respectively) throughout the experiment, compared with the non-supplemented rabbits. Al-Sagheer *et al.* (2017) reported that natural antioxidants can protect intestinal mucosa against oxidative damage and pathogens and limit peristaltic movement in digestive disorders, preventing diarrhoea and enhancing animal performance (Kermauner *et al.*, 2008). Consequently, the Authors postulated that such improvements in growth performance in response to EVOO and LGEO could be linked to the antioxidant activity of their components. On the other hand, significant increases in WBC, lymphocyte, and heterophils counts have been reported in EVOO-supplemented rabbits. According to the Authors, such elevations might be due to activation of gut-associated lymphoid tissue in response to the diet supplemented with EVOO or its cytoprotective activity against free radical-induced injury during heat stress (Khalil *et al.*, 2013).

In order to reduce the environmental stress caused by stocking density in Japanese quail, Bahsi *et al.* (2016) reported that the addition of oleuropein (440 ppm) in the diet increased the body weight gain, feed conversion ratio, and PUFA content, emphasizing that oleuropein was effective in mitigating the negative effects of oxidative stress, especially in a stressed state. This situation can be explained by the addition of essential oils to mixed feed, which then regulate the gastrointestinal tract of animals, increase feed intake, and act as protective agents against bacterial diseases (Dalle Zotte *et al.*, 2016; Elazab *et al.*, 2022). Moreover, intestinal microbial integrity is important in protecting the host from pathogen colonization through multiple mechanisms, including competition for epithelial binding sites, production of bacteriocins, and the strengthening of the intestinal immune response (Burkholder *et al*., 2008; El-Badawi *et al*., 2018).

In recent decades, *in vitro* and *in vivo* studies have provided clear evidence concerning the antimicrobial activity of compounds contained in olive, olive oil, leaves, and vegetation waters (Furneri *et al*., 2004). Thus, many studies have focused on the antimicrobial properties of plant-derived active compounds with the aim of finding and developing new antimicrobial agents that can be transferred to food through the animal diet (Fasolato *et al*., 2015; Branciari *et al*., 2016; Roila *et al.*, 2019; Branciari *et al*., 2021). Studies have demonstrated that the olive-phenol fraction is able to inhibit or delay the rate of growth of a range of bacteria and fungi, and so it can be used effectively as an alternative natural additive in both human and animal diets (Branciari *et al.*, 2021). Furneri *et al*. (2004) showed good antimicrobial activity of oleuropein and hydroxytyrosol against gram-positive and gram-negative bacteria (*Salmonella* sp., *Vibrio* sp., and *Staphylococcus aureus*) (Bisignano *et al*., 2001), as well as the ability of oleuropein to inhibit mycoplasmas (Furneri *et al*., 2002). Obied *et al*. (2008b) reported that the phenolic fraction of OMWW showed antibacterial activity against *S. aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These findings indicate that oleuropein and hydroxytyrosol can be considered an alternative antimicrobial agents for preventing or treating infections.

In growing rabbits, particularly weaners, digestive disturbances are the main cause of important economic losses for rabbit farmers (Marlier *et al*., 2006). The post-weaning period (from 5–8 w of age) is considered a crucial period in which the kits are removed from the mothers. Milk is substituted with solid feed while the kits' immune system and digestive systems (caecal microbiota) are still immature (Gidenne *et al*., 2005; Carabano *et al*., 2006; De Blas *et al*., 2012; Cullere *et al*., 2018).

Table 1 Main effects of dietary supplementation of olive by-products on production and health parameters of rabbits

Parameter	Olive by-products	Dietary level	Overall effect	Reference
Meat oleic acid	Olive Pomace	5%	Improvement	Dal Bosco *et al*. (2012)
Meat oleic acid	Olive Leaves	10%	Improvement	Mattioli *et al*. (2018)
Meat total MUFA	Olive Pomace	5%	Improvement	Dal Bosco *et al*. (2012)
Meat fatty acid profile	Olive Leaves	1%	No effect	Trebusak *et al* (2014)
Body weight at slaughter	Olive Leaves Extract	1.5 ml/kg	Improvement	Younan *et al*. (2018)
Feed efficiency	Olive Leaves Extract	1.5 ml/kg	Improvement	Younan *et al*. (2018)
Meat peroxidation	Olive Pomace	5%	Improvement	Dal Bosco *et al*. (2012)
Meat MDA	Olive Leaves	1%	No effect	Trebusak *et al*. (2014)
Blood MDA	Extra-Virgin Olive Oil	15 g/kg	Improvement	Al-Saagher *et al*. (2017)
Blood CAT	Extra-Virgin Olive Oil	15 g/kg	Improvement	Al-Saagher *et al*. (2017)
Blood GSH-Px	Extra-Virgin Olive Oil	15 g/kg	Improvement	Al-Saagher *et al*. (2017)
White blood cells count	Extra-Virgin Olive Oil	15 g/kg	Improvement	Al-Saagher *et al*. (2017)
Muscle *Pseudomonas spp.*	Olive Mill Waste Water	280 mg/kg	Improvement	Branciari *et al*. (2021)
Muscle *Enterobacteriaceae*	Olive Mill Waste Water	280 mg/kg	No effect	Branciari *et al*. (2021)
Muscle lactic acid bacteria	Olive Mill Waste Water	280 mg/kg	No effect	Branciari *et al*. (2021)

Digestive disorders may be derived from infection, bacteria, or parasites or may be described under the term "non-specific enteritis", in which feeding and stress seem to be the principal agents that provoke different clinical symptoms, intestinal lesions, and diarrhoea (Dalle Zotte *et al*., 2016). Thus, preserving the integrity of gut microbiota and intestinal mucosa adequately through dietary strategies is an important target to reduce productive losses. It was observed by Younan *et al.* (2018) that the supplementation of olive leaf extract (OLE) of up to 1.5 ml/kg of rabbit diets improved the growth performance and increased the final body weight of animals. The Authors supposed that the improvement of rabbit performance may be due to the beneficial effects of OLE phenols in controlling the microbial infections (Aliabadi *et al*., 2012), thus enhancing nutrient digestibility and intestinal absorption. According to Sudjana *et al.* (2009), oleuropein plays a role in regulating the composition of the gastric flora by selectively reducing levels of *C. jejuni* and *S. aureus*. Markin *et al*. (2003) revealed that aqueous OLE 0.6% (*w/v*) in drinking water killed *E. coli*, *P. aeruginosa*, *S. aureus,* and *K. pneumonia*. Moreover, Dalle Zotte *et al*. (2016) refer that olive extract and its active compound, oleuropein, had an antimicrobial effect against pathogens such as *B. cereus, S. aureus, S. enteritidis*, and *Listeria monocytogenes* (Nychas *et al*., 1990). Polyphenolic compounds indirectly decrease the growth of pathogenic microorganisms by increasing the activity of digestive enzymes and reducing toxins within the feed (Wenk, 2002; Tufarelli *et al*., 2016; Younan *et al*., 2018). The overall effects on health and productive parameters of rabbits fed diets including olive by-products are summarized in Table 1.

Conclusions

Because of the increasing trends in consumer demands for functional foods and 'eco-green' products, researchers have directed their attention to the development of reasonable practices that can be adopted to improve the nutritional value and organoleptic properties of meat using bioactive molecules from natural products. Olive by-products represent a valuable source of functional compounds that can potentially be used in animal feeding for food product preservation and meat quality improvement. Furthermore, phenolic extracts from olive by-products could be used as an alternative to synthetic additives that have recently been banned. The reviewed literature, although scarce, confirms that dietary supplementation with olive by-products can satisfy rabbit nutritional requirements as the biocompounds may increase the content of unsaturated fatty acids and the content of substances with antioxidant and antimicrobial activity. Moreover, the literature demonstrates a negative, linear relationship between plasma redox status and the development of detrimental oxidative processes in the muscle, highlighting the influence of phenolic compounds on meat stability. Thus, it can be concluded that dietary supplementation with olive by-products can be a feasible approach in rabbit farming to support performance, reduce costs, and respond to the consumers request for high quality, healthy, safe, and friendly products. Further research is indicated to explore their biological action in organic systems and to produce alternative feeding strategies that enrich meat in precious compounds and improve the oxidative stability of meat products.

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Author contributions

All the authors approved the final version of the manuscript. CL, MS, VL, and VT: conceptualization, writing-review & editing, writing-original draft.

Data availability

Data are contained within the article.

Conflict of Interest Declaration

The authors declare no conflicts of interest.

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