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Review article

Use of polyphenol-rich grape by-products in monogastric nutrition. A review

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ABSTRACT

Plants and their biologically active chemical constituents present numerous opportunities for improving animal production by inclusion in the diet. In recent years, interest has grown in the antioxidant and antimicrobial properties of a number of polyphenols found in different plants. The by-products of the wine industry (grape pomace, skin and seeds) and wine polyphenol extracts contain a wide range of bioactive compounds. However, studies on grape by-products are very limited, despite their richness polyphenolic substances. In this context, the purpose of this review is summarize recent advances of research in grape by-products including the phenolic composition, mechanism of intestinal and hepatic conjugation, plasma transport and elimination in bile and urine, and biological activities such as antioxidant and antimicrobial effect. Given their antioxidant activity, the inclusion of these by-products in feed rations would not only enhance the oxidative stability of the meat and reduce the amount of additives like vitamin E but also improve meat quality through direct addition of these natural antioxidants, thereby helping to meet consumer demand for healthier meat products. With respect to antimicrobial activity, they enhance the growth of specific beneficial bacteria strains in the intestinal tract while competitively excluding certain pathogenic bacteria.

Contents

Abbreviations: PUFA, polyunsaturated fatty acid; SFA, saturated fatty acids; GP, grape pomace; GS, grape seed; GSE, grape seed extract; EPA, extractable polyphenols; NEPA, non-extractable polyphenols; PB1, procyanidin B1; PB2, procyanidin B2; MDA, malondialdehyde; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; ABTS, [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)]; PG, propyl gallate.

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1. Introduction

By-products from the winemaking industry are widely available in Mediterranean countries. The grape is the world's largest fruit crop, with an annual production of more than 67 million tonnes (FAO STAT, 2010). It is estimated that around 20% of the total weight of grapes used for wine is made up of grape pomace (GP), the solid residue left over after the juice is extracted from the grapes. This by-product, consisting of the seed, skin, and stem, is used in the production of ethanol by fermentation and distillation and in the extraction of tartaric acid. With respect to animal feed, the nutritional value of these by-products is relatively low. It is estimated that some 3% of production is used mainly in the manufacture of maintenance feed for ruminants and to provide some of the dietary fibre needs of rabbits (Nicodemus et al., 2007). GP has also been used to extract the oil contained in the grape seed.

Grape pomace is particularly rich in a wide range of polyphenols. Formerly known as tannins, these polyphenols were considered to be anti-nutritional factors as their presence in certain ingredients, such as legumes, sunflowers and sorghum had negative effects on animal nutrition. The major limitations of using GP in monogastric feed are the high level of lignified cell wall fraction and the high tannin content. However, studies carried out *in vivo* and *in vitro* over the last few years have shown the beneficial effects of administering these bioactive compounds because of their antioxidant and antimicrobial activity (Alonso et al., 2002; Torres et al., 2002; Viveros et al., 2011).

The use of polyphenols is recommended to limit lipoperoxidation and preserve animal health and product quality (Georgiev et al., 2014). Lipid peroxidation is a matter of great interest, both for the food industry and for consumers, as it leads to the development of unpleasant smells or tastes in food products, which may become potentially toxic. Meat from domestic animals is particularly exposed to lipoperoxidation processes, not only because it is at higher risk of oxidative stress but also for reasons of diet. The inclusion of polyunsaturated fatty acids (PUFA) in the diet is a generally accepted practice for raising the energy content of the feed or producing animal products with a fatty acid composition that better meets nutritional guidelines (Bourré, 2005). However, a high level of polyunsaturation accelerates oxidative processes, leading to a deterioration in the flavour, texture and nutritional value of the meat (Mielnik et al., 2006). Manipulation of the gut functions and microbial habitat of domestic animals with feed additives has also been recognized as an important tool for improving growth performance and feed efficiency. The increasing antimicrobial resistance of pathogens isolated from humans and animals, combined with the ban on the use of antibiotics as feed additives, has hastened research into alternative options for more antimicrobial efficient in animal production (Hughes et al., 2005).

In short, increased efforts are now being directed towards a broader revaluation of polyphenol-rich residues from wine processing for obtaining high-value co-products (GP, seeds, skin and grape polyphenols). The purpose of this review is to understand the mechanism of their biological activities with a view to exploring the potential application of these bioactive compounds, and to outline recent scientific advances supporting their beneficial qualities, bioavailability and use as ingredients or additives in monogastric nutrition.

2. Classification, chemical composition and distribution of phenolic compounds in grapes

The winemaking industry produces large quantities of waste residue consisting mainly of solid by-products, including GP (seeds, skin and stems). The seed and skin represent 38–52% and 5–10% of GP, respectively, on a dry matter basis. Chemical composition of the GP, seed and skin is shown in Table 1. In general, the content of highly lignified fibre in grape by-products is high, which limits their use in monogastric animals. Samples reveal that the nutritional composition varies widely, depending on the variety of grape, the location and the conditions of fertilization. These residues also have high polyphenol content (Table 2).

Polyphenols are compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings (Vermerris and Nicholson, 2006). They are classified according to the number of phenolic groups they contain and the structural elements attached to these rings, which include phenolic acids, flavonoids, stilbenes and lignans. The largest and most widely studied group of polyphenols are the flavonoids. Flavonoids are divided into seven subclasses based on their molecular structures: flavones (apigenin and luteolin), flavanones (hesperidin, naringenin and eriodictyol), flavonols (kaempferol, quercetin, myricetin), isoflavones (daidzein, genistein and glycitein), anthocyanidins/anthocyanins (cyanidins, delphinidins, *etc.*), flavanols (catechins and procyanidins) and chalcones (Manach et al., 2004).

The phenolic composition of GP, seeds and skin is shown in Table 3. In the grape, the compounds found in the greatest proportion are the flavanols, which include simple monomers of catechin and its isomer epicatechin, as well as oligomeric proanthocyanidins (from 2 to 5 units) and polymers (more than 5 units), commonly known as condensed tannins (Crozier, 2003). Unlike other classes of flavonoids, flavanols are not glycosylated in foods. Catechin is the most abundant of all the flavanols. It is found in the seeds and skin of the grape, although traces of monomers and dimers have also been found in grape pulp. Phenolic acid, mainly hydroxycinnamic (in the form of esters of tartaric acid) is found in the skin and the

Table 1
Chemical composition of grape pomace, seed and skin (g/kg as fed basis).

	Grape pomace	Seed	Skin
Dry matter	900–930[1]	910–930[1]	801–930[1]
Protein	112–138[1,9]	93–146[1,2,6]	110–138[1,2,6,8]
Fat	56–117[1,3]	95–111[1,2,6]	32–63[1,2,6,8]
Ash	24–58[1,3]	29[1,10]	62–75[1,8]
Fibre	325–563[1,8,9]	414[1]	306[1]
Neutral detergent fibre	542–708[1,3,5]	503–670[1,2,6,10]	243–704[1,2,6,7]
Acid detergent fibre	480–704[1,3,5]	454–570[1,2,6]	193–490[1,2,6]
Acid detergent lignin	307–475[1,3,5]	214–437[1,2,6,10]	283–437[1,2,6]
Condensed tannins			
Free	16–38[3]		
Fibre-bound	19–34[3]		
Protein-bound	56–131[3]		
Total	91–203[3]		
Minerals			
Ca	5–7 [1,2]	5–7[1,4]	41–70[1,2]
P	2–3[1,2]	2–4[1,4]	23–29[1,2]
Fe[a]	64–185[1,2]	45–120[1,4]	117–398[1,2]
Cu[a]	65–124[1,2]	6.4–20[1,4]	23–124[1,2]
Zn[a]	12–18[2]	9.5–15.0[1,4]	18–12[2]
Mn[a]	13–17[2]	11.3–21[1,4]	13–17[2]
Vitamin E[a]		4.0–22.8[1,4]	

Based on data obtained from: [1]FEDNA Tables (2010); [2]Spanghero et al. (2009); [3] Molina-Alcaide et al. (2008); [4]Lachman et al. (2013); [5]Eraso et al. (1991); [6]Guerra-Rivas et al. (2013); [7]Tortuero et al. (1994); [8]Deng et al. (2011); [9]Llobera and Cañellas (2007); [10]Correddu et al. 2015.

[a] Expressed as mg/kg dry matter.

Table 2
The phenolic compounds in different parts of grape and its products.

Source	Phenolic compounds
Seed	Gallic acid, catechin, epicatechin, dimeric procyanidin, proanthocyanidins.
Skin	Proanthocyanidins, prodelphinidins, ellagic acid, myricetin, quercetin, kaempferol, trans-resveratrol.
Stem	Rutin, quercetin 3-*O*-glucoronide trans-resveratrol.
Leaf	Myricetin, ellagic acid, kaempherol, quercetin, gallic acid.
Raisin	Hydroxycinnamic acid, hydroxymethylfurfural.

Adapted from Xia et al. (2010).

pulp. Finally, flavonols, stilbene derivatives and anthocyanins are found in the skin. The polyphenol composition of each part of the GP also varies depending on the variety of grapes and is influenced by growing condition, climate, maturity and fermentation time (Rodríguez-Montealegre et al., 2006).

With regard to the extraction technique, polyphenols can be divided into two groups: those that can be extracted from the feed matrix by aqueous-organic solvents (extractable polyphenols or EPAs), and those that remain in the extraction residue, known as non-extractable polyphenols (NEPAs). EPAs include low- or intermediate-molecular weight polyphenols (dimers to decamers), while NEPAs are mainly structures linked to the protein and polysaccharides of the dietary fibre matrix, which cannot be extracted either by the action of digestive enzymes or by the usual aqueous acidic methanol–acetone

Table 3
Phenolic composition of main phenolic content in grape pomace, seeds and skins.

	Grape pomace	Seeds	Skins
Total phenol content	19–40.5[2]	36.6–88.7[2]	20.2–52.3[2]
Total tannins	39.1–105.8[2]	62.3–167.8[2]	44.9–73.0[2]
Phenolic acids	0.03–8.31[10]	0.10–0.11[10]	0.17–8.23[10]
Catechin	0–0.3[1,9]	2.14–2.42[2,3]	0–0.3[2,5,6,7]
Epicatechin	0–0.2[1,9]	0.88–1.60[2,3,5]	0–0.13[2,5,6,7]
Epigallocatechin	0–0.05[4]	0.05[4]	ND
Epigallocatechin-gallate	0–0.007[3]	0.06–0.07[4]	ND
Epicatechin gallate	0.003[1]	0.25–0.31[4]	0.04[7]
Procyanidin B1	0.11–0.60[1,7,8]	0.14–0.17[3,5]	0.18–0.6[5,7,8]
Procyanidin B2	0.01–0.84[7,8]	0.04–0.18[3,5]	0.01–0.84[5,7,8]
Anthocyanin[a]	11.47–29.82[10]	ND	11.47–29.82[10]
Total flavonols	0.03–0.63[10]	0.02–0.05[10]	ND

All values are given in g/kg dry matter. [1]Chamorro et al. (unpublished data); [2]Ky et al. (2014); [3]Guendez et al. (2005); [4]Souquet et al. (2000); [5]Rodríguez-Montealegre et al. (2006); [6]Yilmaz and Toledo (2004); [7]De Freitas et al. (2000); [8]Mateus et al. (2001); [9] Arts et al. (2000); [10]Pinelo et al. (2006); ND, non-detected.

[a] Anthocyanin is exclusive for red grapes.

solvents (Serrano et al., 2009). The most common way of separating these compounds from the extraction residue is by using hydrolysis reactions, generally acid, at high temperatures. This not only allows the polyphenols attached to the vegetable matrix to be released but produces the depolymerization of high molecular weight compounds into more simply structured compounds that can be quantified using known models.

Identification of the polyphenolic compounds present in each of the fractions has generally been carried out using simple spectrophotometric methods based on different chemical principles. The Folin–Ciocalteu method has traditionally been used to quantify the total number of polyphenols (Singleton et al., 1999), and the vanillin method to determine condensed tannins (Okuda et al., 1989). However, due to interferences created by factors like the nature of the dissolvent, the pH value and the presence in the sample of other compounds that absorb UV–vis, such as fats, vitamins and amino acids, these methods are not very accurate. In recent years, most work on the analysis of polyphenols has been based on the use of methods of chromatographic separation (particularly high-performance liquid chromatography or HPLC), followed by structural characterization using mass spectrometry (MS). This methodology (HPLC–MS) is currently used to quantify extractable polyphenolic compounds. However, the quantification of more complex compounds, such as non-extractable polyphenols, continues to be based on the use of less accurate spectrophotometric methods.

3. Digestion, absorption and metabolism of grape polyphenols

To explain the biological effects of polyphenols, it is assumed that they are bioavailable and are effective in reaching target tissues. It is therefore important to fully understand how they are absorbed, metabolized and eliminated from the body. There is considerable controversy surrounding current studies on the absorption and metabolism of polyphenols and results are therefore inconclusive. Studies on absorption are made difficult by the molecular complexity of the extracts or polyphenol-rich feed owing to factors like their level of polymerization and conjugation with other phenols. Most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in their native form. These substances must be hydrolysed by endogenous enzymes or microbiota before they can be absorbed. Once absorbed, polyphenols are recognized by the body as xenobiotics, and their bioavailability is therefore relatively low in comparison to micro- and macronutrients.

The metabolization of polyphenols takes place through a sequence of reactions common to all of them. This is similar to a metabolic detoxication to reduce their potential cytotoxic effect by increasing their hydrophilicity and facilitating urinary or biliary elimination (Manach et al., 2004). It is the chemical structure of polyphenols, rather than the concentration, that determines the rate and extent of absorption and the nature of the metabolites circulating in the plasma. Depending on their degree of structural complexity and polymerization, these compounds may be readily absorbed in the small intestine (*i.e.*, low-molecular-weight polyphenols such as monomeric and dimeric structures) or reach the colon almost unchanged (oligomeric and polymeric polyphenols) (Monagas et al., 2010). It has been estimated that only 5–10% of the total polyphenol intake may be absorbed in the small intestine. After absorption, these less complex polyphenol compounds may be subjected to hydrolysis and biotransformation in the enterocytes and then the hepatocytes, resulting in a series of water soluble conjugate metabolites (methyl, glucuronide and sulphate derivatives) being rapidly released into the systemic circulation for further distribution to organs and excretion in urine (Manach et al., 2004). A proportion of these metabolites enters the duodenum by means of the bile and is subsequently hydrolysed by bacterial enzymes (mainly β-glucuronidase) in the large intestine. This enterohepatic recycling may lead to a longer presence of polyphenols within the body. However, the remaining polyphenols (90–95% of the total polyphenol intake) may accumulate in the large intestinal lumen where, together with conjugates excreted into the intestinal lumen through the bile, they are subjected to the enzymatic activities of the gut microbial community, generating metabolites such as aromatic acids (hydroxyphenylacetic, phenylpropionic, phenylbutyric acids, phenyl valerolactones and others) (Selma et al., 2009; Sánchez-Patán et al., 2012). All these microbial-derived phenolic metabolites may be absorbed or excreted in the faeces. When absorbed, they reach the liver through the portal vein where they may be further subjected to extensive metabolism (including glucuronidation, methylation, sulphation or a combination of these) until they finally enter the systemic circulation and are distributed to the organs or eliminated in the urine. The gut microbiota are therefore responsible for the extensive breakdown of the original polyphenolic structures into a series of low-molecular-weight phenolic metabolites which, being absorbable, may actually be responsible for the biological activity derived from polyphenol-rich food consumption, rather than the original compounds found in foods. The concentrations of polyphenols reached after their consumption varies significantly according to the nature of the polyphenol and the food source. Plasma concentrations in intact flavonoids rarely exceed 1 μM and the maintenance of a high polyphenol concentration in plasma requires repeated ingestion over time; in fact, maximum concentrations are most often reached 1–2 h after ingestion (Manach et al., 2004). Polyphenols and their derivatives are eliminated chiefly in the urine and the bile (Fig. 1).

Until now, research on the digestibility of polyphenols from grape by-products in domestic animals has been lacking, as have studies on their effect on the digestibility of other nutrients. Recent work carried out in our laboratory shows that concentrations of up to 6% of grape pomace (GP) and 0.25% of grape seed extract (GSE) can be used in chicken feed without any modification in performance, the size of the digestive organs or the ileal digestibility of protein and amino acids (Brenes et al., 2008; Chamorro et al., 2013). Studies using spectrophotometric techniques on the digestibility in birds of non-extractable polyphenols present in GP (Brenes et al., 2008) showed that the ileal and faecal digestibility of hydrolysable polyphenols (56% and 73%, respectively) was greater than that of condensed tannins (14% and 47%, respectively). On the other hand, greater digestibility of the total extractable polyphenols in the birds' diets was observed when commercial polyphenolic

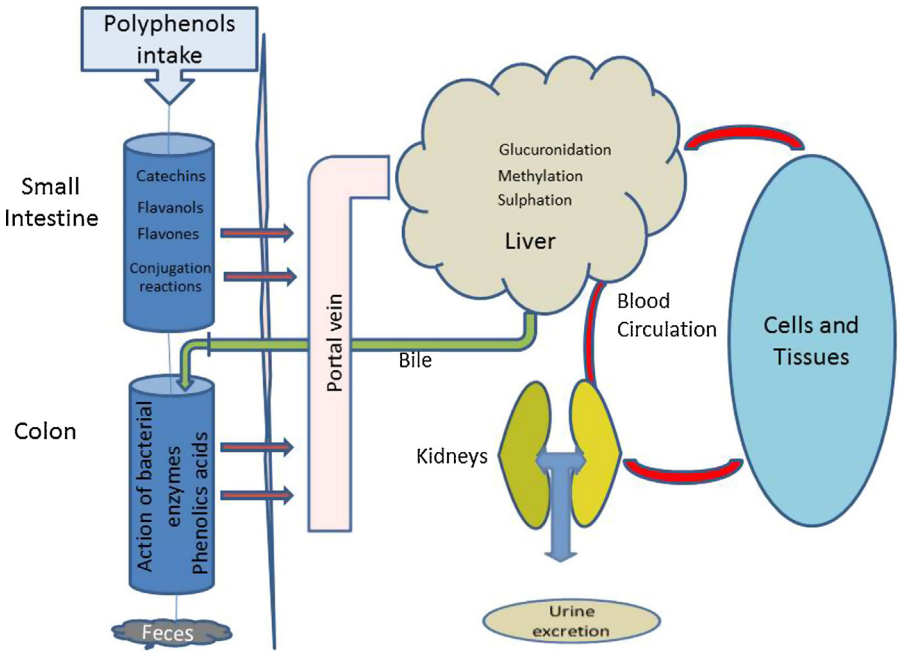

Fig. 1. Schematic description of metabolic fate of dietary polyphenols.

grape extracts (GE) were used (Brenes et al., 2010), with values of up to 69% obtained at faecal level. Liquid chromatography techniques in combination with mass spectrometry, which allows the different polyphenolic compounds present in grape by-products to be identified and quantified, have recently been used to demonstrate that extractable polyphenols are highly digestible in birds at both ileal and faecal level (Chamorro et al., unpublished). The results also show that digestibility of these compounds depends on their degree of polymerization (which is greater in monomers [catechin and epicatechin] than in dimers [B1 and B2 procyanidins]), and their degree of esterification (greater in free compounds than in those esterified with gallic acid). It has also been shown that the inclusion of polyphenolic grape extracts at concentrations of 5 g/kg in birds' diets can reduce the digestibility of other nutrients like fat (Brenes et al., 2008), protein and certain amino acids, such as proline and cystine (Chamorro et al., 2013).

The bioavailability of procyanidin is largely influenced by its structure. For this reason, there is increasing interest in how dietary flavonoids are broken down into simple phenolic compounds. Strategies to encourage hydrolysis of the more complex polymeric structures of polyphenols have been studied *in vitro* with the aim of improving the digestibility of polyphenols present in grape by-products. These have included the study of thermal treatment (furnace and autoclave) and the addition of enzymes (pectinase, cellulose and tannase) to degrade or convert the polymeric structures into compounds with a lower molecular weight (monomers and oligomers). Results reported by Chamorro et al. (2012a) showed that wet heating conditions increased hydrolysis of procyanidins. The effectiveness of the thermal treatment varied according to whether polyphenols were present in free form (GSE) or whether they were bonded or linked to other structures (GP). Furnace thermal treatment did not modify the polyphenol compounds of these grape products. However, the effect on individual compounds in GSE was more severe with autoclave treatment, leading to extensive hydrolysis of catechin, epicatechin, gallocatechin, PB1 and PB2. In the case of GP, autoclave treatment increased gallic acid, gallocatechin and epigallocatechin. These modifications suggest that during autoclave treatment the more highly polymerized molecules seem to be changed to relatively less polymerized molecules. At the same time, the addition of commercial cell-wall-hydrolysing enzymes (pectynolitic and cellulolytic activities) was used to release cell wall complex polysaccharides present in GP, facilitating the release of certain nutrients entrapped by the cell wall. Tannase or tannin acyl hydrolase (EC, 3.1.1.20) was also used to catalyze hydrolysis of the ester and depside bonds present in hydrolysable tannins or gallic esters in GP and GS (Chamorro et al., 2012b). The results obtained in this study demonstrate that the use of pectinase and tannase in both grape by-products and pectinase in GP changed the galloylated form of catechin to its free form, releasing gallic acid. Pectinolytic enzymes also broke down the GP plant cell-wall matrix, releasing monosaccharides and facilitating polyphenol extraction.

The applicability of these findings with enzymes *in vitro* needs to be supported by experiments *in vivo*. The inclusion of enzymes (carbohydrases and tannase) in chicken diets containing GP (5% and 10%) modified the polyphenol polymeric structures present in the ileal content, with increased concentrations of gallic acid, catechin and epicatechin in birds fed carbohydrases, and of gallic acid, catechin, epicatechin, procyanidins PB1 and PB2 and epicatechin gallate with the addition of tannase. These findings show that the inclusion of enzymes in diets containing GP increased the amount of total polyphenol

released in the intestine, although this effect was not accompanied by an increase in the birds' performance (Chamorro et al., 2015).

4. Application of biological activities of grape polyphenols in animal nutrition

The biological effects of polyphenols have been extensively studied *in vitro* and in animal models (Yu and Ahmedna, 2013). However, data from domestic animals is still very limited. Recent research has stressed the importance of by-products from wine processing as plant materials that are not only particularly rich in polyphenols but have a wide range of biological activities. When applied to monogastric nutrition, they offer promising possibilities as tools for improving certain aspects of meat and milk quality and for modifying intestinal microbiota (Tables 4 and 5).

4.1. Antioxidant activity

Antioxidant activity is the most notable bioactivity of phenolic compounds from GP (Xia et al., 2010; Georgiev et al., 2014). *In vitro* and *in vivo* studies have shown that flavonoids present in wine by-products behave like free radical scavengers by acting as powerful antioxidants and metal chelators. This inhibits formation of the superoxide ion and indirectly inhibits redox-sensitive transcription factors and pro-oxidant enzymes (Puiggros et al., 2005). They also activate antioxidant enzymes, reduce α-tocopherol radicals (tocopheroxyls), inhibit oxidases and increase levels of uric acid and substances of low molecular weight. The antioxidative properties of polyphenols result mainly from their ability to donate hydrogen from hydroxyl groups positioned along the aromatic ring in order to terminate the free-radical oxidation of lipids and other biomolecules (Foti et al., 1994). Among the phenolics, monomeric forms are less potent as hydrogen-donating radical scavengers than polymeric phenols (Fiueroa-Espinoza and Villeneuve, 2005).

At low concentrations, antioxidants are substances that retard the oxidation of easily oxidable biomolecules, such as lipids and proteins in meat products, thus improving the shelf life of products by protecting them against deterioration caused by oxidation. In response to recent demands for natural products and the willingness of consumers to pay significant premiums for natural foods (Sebranek and Bacus, 2007), the meat industry is actively seeking natural solutions to minimize oxidative rancidity and increase product shelf-life. Oxidative stress is commonly defined as an imbalance between oxidants and antioxidants at cellular level. In animals, oxidative stress may occur as a consequence of nutrition, including the contamination of feed with fungal toxins, high environmental temperatures and several pathological conditions, such as increased activity of the immune system (infection, vaccination), pulmonary hypertension, ascites and coccidiosis (Frankič et al., 2008; Lin et al., 2006; Georgieva et al., 2006).

4.1.1. Supplementation of grape by-products to the diet

Nutrition has a strong impact because an insufficient intake of antioxidants, a high intake of pro-oxidants, or both, may lead to oxidative stress. Animal nutrition is currently evolving towards n-3 polyunsaturated fatty acid (PUFA) to improve animal fat healthfulness but this nutritional strategy has been associated with an increase in lipoperoxidation in subcutaneous and intramuscular lipids. Dietary PUFA are a group of pro-oxidants known to increase oxidative stress *in vivo* in pigs (Salobir et al., 2005), chicks (Gao et al., 2010) and hens (Cherian and Hayat, 2009). Increasing the level of unsaturation in the muscle membrane by dietary manipulation increases the susceptibility of meat to oxidative deterioration during storage, the consequence being a reduction in both flavour and nutritional value (Enberg et al., 1996). Endogenous defence mechanisms are inadequate for complete prevention of oxidative damage. Vitamin E is the antioxidant most commonly used in animal nutrition but this has certain drawbacks, including its synthetic origin, its limited bioefficiency when $n = 3$ PUFA intake is too high (Allard et al., 1997), its potential pro-oxidant action (Mukai et al., 1993), and its non-homogeneous distribution between the tissues. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used as antioxidants in meat and animal products, but these have come under scrutiny because of their potential toxicological effects (Karre et al., 2013). There is a growing interest in the nutritional aspect of polyphenolic compounds in light of their antioxidant capacity and they may become an important alternative as a partial substitute for vitamin E in animal diets. The antioxidant potential of grape seed polyphenols is 20 times higher than vitamin E and 50 times higher than vitamin C (Carpenter et al., 2007).

Natural antioxidants can be applied either through dietary or technological strategies to reduce or prevent oxidative processes in meat. The optimum dose of inclusion of polyphenols in animal diets is difficult to define due to the different composition of phenolic compounds present in these by-products. Nevertheless, the use of plant-rich polyphenols seem to be a promising strategy for improving products quality. In dietary manipulations, antioxidants are introduced into the muscle *via* the animal feed. Various authors have reported that inclusion of natural antioxidants in animal diets not only slows down oxidation, but also greatly improves meat quality when compared to diets with no antioxidants (Table 4). It has been shown that the addition of polyphenolic grape extract in monogastric diets, especially oligomeric flavanols, enhances oxidative stability in chicken and turkey meat (Lau and King, 2003; Rababah et al., 2006).

Studies carried out and published by our research group (Goñi et al., 2007; Brenes et al., 2008, 2010) also showed that increasing concentration of GP up to 60 g/kg and GSE up to 3.6 g/kg improved the antioxidant activity of the diet, ileal content and faeces. Likewise, we also observed an enhance oxidative stability in chicken meat products (TBARS, thigh and breast) during the refrigeration process. Similar increment was obtained when vitamin E was added to the rations. The concentration

Table 4
Feeding value of grape by-products.

Specie	Grape by-product	Dose	Effect	References
Chickens	GSE	10 g/kg	No effect on growth performance.	Hughes et al. (2005)
	GSE (296 g GAE/kg and 148 g cyanidin equivalent/kg)	0.01 g/kg	Improved growth performance.	Juin et al. (2007)
	GP (48.6 g GAE/kg and 160.4 g anthocyanidins/kg)	5–30 g/kg	Reduction of meat lipid oxidation improving vitamin E status. No effect on growth performance.	Goñi et al. (2007)
	GP (48.6 g GAE/kg and 160.4 g anthocyanidins/kg)	15–60 g/kg	Digestibility of polyphenols. Determination of ileal and faecal digestibility of hydrolizable (56–73%) and condensed tannins (14–47%). Reduction of meat lipid oxidation. No effect on growth performance.	Brenes et al. (2008)
	GSE (10% monomer and 61.32 oligomer)	0.005–0.08 g/kg	Reduction of oxidation stress in coccidiosis.	Wang et al. (2008) Naidoo et al. (2008)
	GSE (296 g GAE/kg and 148 g cyanidin equivalent/kg)	0.6–3.6 g/kg	No effect on growth performance. Improved polyphenols digestibility. Antioxidant effect of diet and excreta.	Brenes et al. (2010)
	Resveratrol (500 mg/kg)	0.2, 0.4 g/kg	No effect on growth performance. Reduced serum and egg TBARS values and increased serum Vitamin E concentration in quail.	Sahin et al. (2010)
	GP and GSE	GP: 60 g/kg; GSE: 7.2 g/kg	Modified the gut morphology and intestinal microbiota.	Viveros et al. (2011)
	GSE (890 mg/kg polyphenols containing 31.1 mg/g catechins and 112 mg/g oligomeric proanthocyanidins)	0.01–0.2 g/kg	No effect on plasma and lipid oxidation.	Vossen et al. (2011)
	GSE (296 g GAE/kg and 148 g cyanidin equivalent/kg)	0.025–5 g/kg	No effect on growth performance (up to 2.5 g/kg). Reduction of free plasma iron concentration.	Chamorro et al. (2013)
	GP (48.6 g GAE/kg and 160.4 g anthocyanidins/kg)	50, 100 g/kg	No effect on growth performance. Reduced meat lipid oxidation similar to vitamin E. Increased PUFA content of thigh meat.	Chamorro et al. (2015)
	GP concentrated (38.5 g/kg TP and 27 g/kg proanthocyanidins)	0.025, 0.050, 0.075 g/kg	Replacing vitamin E. No effect on growth performance. Reduced meat lipid oxidation.	Iqbal et al. (2014)
	Resveratrol	0.2, 0.4, 0.6 g/kg	Improved growth performance and reduced oxidative stress black-bone chickens.	Liu et al. (2014)
Pigs	Extract containing polyphenols including GSE	2 g/kg	No influence on antioxidant content and lipid oxidation of free-range rearing pig meat.	Gonzalez and Tejeda (2007)
	GSE (865 g GAE kg)	0.1, 0.3, 0.7 g/kg	No effect on oxidative stability and quality of raw and cooked meat.	O'Grady et al. (2008)
	Fermented GP (62.1 g GAE/kg)	30 g/kg	Improved growth performance and N digestibility. Modified the fatty acid pattern in subcutaneous fat.	Yan and Kim (2011)
	GSE (34, 46 and 65 mg/g of GA, C and EC)	*In vitro* (250 μg/ml)	Modified the pattern of starch fermentation in ileal and faecal microbiota.	Wang et al. (2012)
	GP Fermented diets	100–200 g/kg	Increased the number of beneficial bacteria. Decreased VFA emission in faeces.	Cho et al. (2012)
	GP and grape marc meal extract (85 g GAE/kg)	*In vitro* (0.02–2 μg/ml) *In vivo* 20 g/kg	Prevention of intestinal inflammatory processes. Inhibition of inflammatory processes in intestinal epithelium model and decreases activities of the oxidative stress-responsive transcription factors NF-kB and Nrf2 in the duodenal mucosa in weaned pigs.	Gessner et al. (2012, 2013)
	GS and grape marc meal extract (85 g GAE/kg)	10 g/kg	Improved the gain feed-ratio in weaned pigs. Modification of intestinal microbiota and anti-inflammatory effect.	Fiesel et al. (2014)
	GSE (Low molecular weight polyphenols)	10 g/kg	Reduced *E. coli* induced diarrhoea in weaned pigs.	Verhelst et al. (2014)
	Extract containing polyphenols including GSE	1 g/kg	No differences in microbial count in faeces and caecum in weaned pigs. Reduced plasma TBARS values.	Zhang et al. (2014)

Table 4 (*Continued*)

Specie	Grape by-product	Dose	Effect	References
	Dehulled grape seed meal	100 g/kg	No effect on growth performance and carcass quality in fattening rabbits.	Cavani et al. (1988)
Rabbits	Grape pulp meal	280 g/kg	No effect on growth performance. Increase of caecal volatile fatty acid and intestinal microflora. Reduction of plasma Cu. Increased caecal number of total aerobic and sulphite-reducing bacteria in fattening rabbits.	Tortuero et al. (1994)
	GP	100–300 g/kg	Linear decrease of gain:food ratio. Increase digestible crude protein utilization. Reduction of caecal ammonia and volatile fatty acid. Digestibility, efficiency of utilization and caecal fermentation.	Motta-Ferreira et al. (1996)
	Defatted grape seed meal	25–75 g/kg	No effect on growth performance and digestibility of nutrients in fattening rabbits and lactating does using 25 g/kg.	Nicodemus et al. (2007)
	GSE and GPE	2 g/kg	Activation of antioxidant status and reduction of plasma lipid oxidation in hypercholesterolemic rabbits.	Choi et al. (2010)

GP, grape pomace; GS, grape seed; GSE, grape seed extract; GPE, grape peel extract.

Table 5
Applications of grape by-products as natural antioxidants in meat and meat products.

Plant extract	Concentration	Meat product	Storage conditions	Effects	References
GSKE	0.2 g/kg	Pork patties	Vacuum packaged, 4.5 °C, 10 days	Reduced TBARS and hexanal values.	Nissen et al. (2004)
GPE	0.1 g/kg	Frozen fish muscle	−10 °C, 5–6 months	Inhibited oxidation by flavanol oligomers.	Pazos et al. (2005)
GSE	0.4, 0.8, 1.6 g/kg	Cooked turkey breast	Vacuum packaged, 13 days	Inhibited oxidative rancidity and volatile compounds formation.	Mielnik et al. (2006)
GSE	2.5 g/kg	Cooked chicken breast	4 °C, 6 and 12 days	Reduced TBARS values.	Rababah et al. (2006)
GSE	1, 10 g/kg	Ground raw, cooked pork	Refrigerated and frozen storage	Inhibited primary and secondary oxidation products.	Brannan and Mah (2007)
GSE	0.1–0.2 g/kg	Cooked pork patties	Patties overwrapped in PVC, 4 °C, 8 days	Reduced oxidative rancidity and reduced visual green discoloration in beef patties.	Rojas and Brewer (2007)
GSE	0.1–0.2 g/kg	Raw pork patties	Vacuum packaged, −18 °C, 4 months	Reduced oxidative rancidity. GPS showed best antioxidant activity based on TBARS values.	Rojas and Brewer (2008)
GP	24 g/kg	Horse mackerel	−20 °C, 6 months	Inhibition of lipid oxidation and rancidity.	Sánchez-Alonso and Borderías (2008); Sánchez-Alonso et al. (2008)
GSE	10 g/kg	Raw and cooked breast and thigh	−4 °C, 4, 8, 12 days −18 °C, 14 days	Reduced flavour and odour in breast. Altered colour in breast and thigh.	Brannan (2009)
GSE	0.2 g/kg	Cooked frozen pork patties	Cooked patties overwrapped in PVC, 18 °C, 6 months	Reduced TBARS values. GSE showed more antioxidant activity than oleoresin rosemary, water soluble oregano extract, BHT and BHA.	Sasse et al. (2009)
GP	5, 10, 15, 20 g/kg	Raw and cooked chicken hamburgers	Over wrapped in PVC, 4 °C for 3, 5 and 13 days	Improved the oxidative stability.	Sayago-Ayerdi et al. (2009)
GSE	2.5 g/kg	Cooked chicken breast	5 °C, 6,12 days	Reduced TBARS values.	Shirahigue et al. (2010)
GS and GSKE	0.06 g/kg	Raw and cooked thigh chicken meat	Vacuum packaged, 18 °C, 9 months	Reduced TBARS values. Darkening and lower intensity of red and yellow colour.	Selani et al. (2011)
GSE	20 g/kg	Mackerel minced muscle	−18 °C, 3 months	Reduced lipid hydroperoxides and TBARS values.	Ozen et al. (2011)
GSE	1 g/kg	Raw pork patties	2 °C, 1, 6, 13, 20 days	Reduced lipid oxidation and limited colour deterioration. Decreased total viable count (lactic acid bacteria, *Pseudomonas* and psychotropic aerobic bacteria).	Lorenzo et al. (2014)

GSKE: grape skin extract; GPE, grape pomace extract; GSE, grape seed extract; GP, grape pomace; GS, grape seed.

of vitamin E in the liver also increased with the addition of GP, which could be due to a vitamin E saving effect. A reduction in α-tocopherol deposition in chicks fed unsaturated diets was also reported by Surai and Sparks (2000) and Sijben et al. (2002). The increased vitamin E content in the liver with the inclusion of GP could be due to the saving effect of GP in the intestine. Less vitamin E would be destroyed through oxidation, resulting in greater amounts of the vitamin being absorbed. It has also been reported in rat and human models that, owing to their 1-electron reduction potentials, polyphenols may save vitamin E to delay lipid oxidation and regenerate tocopherol (Frank, 2005).

The inclusion of GP and GSE in chicken diets significantly improved oxidative stability (TBARS) and radical scavenging capacity (ABTS) in raw breast meat and cooked chicken patties (Sayago-Ayerdi et al., 2009a,b; Selani et al., 2011). Similar results have been reported by Brannan (2008) and Lau and King (2003) in chicken thigh meat during refrigerated storage and by Mielnik et al. (2006) in turkey meat. Sahin et al. (2010) and Liu et al. (2014) reported that the inclusion of resveratrol in quail diets enhanced the antioxidant activity status of birds and eggs and reduced oxidative stress in heat-stressed chickens by increasing serum growth hormone concentrations and modulating the expression of heat shock genes in organs of the immune system.

A reduction of plasma TBARS values (Zhang et al., 2014) and a modification in the fatty acid pattern in subcutaneous fat (Yan and Kim, 2011) have also been reported in pigs fed diets containing a plant extract including GSE (2 g/kg) and fermented GP diets (30 g/kg), respectively. However, the addition of GSE (0.7 g/kg) did not affect the oxidative stability and quality of raw and cooked pig meat (O'Grady et al., 2008).

All these studies, mainly in chickens, suggest that polyphenols present in grape by-products are absorbed, distributed and retained and remain functional at sufficient levels to contribute to PUFA protection in membranes and modulate antioxidant activity in muscle tissue. These bioactive compounds could therefore reduce the amount of additives like vitamin E but also improve vitamin E status. Increases in the price of this vitamin, resulting from the rising cost of raw materials and energy in recent years, together with the potential environmental impact of the manufacture of vitamin E and the growing demand for this functional antioxidant in the feed industry, ensure the need for research into cheaper but functionally equivalent products as an alternative to this vitamin.

4.1.2. Direct addition of grape by-products to meat products

In recent years, much attention has been paid to developing meat and meat products with physiological functions to promote healthy conditions and prevent disease. Technological strategies involve the application of antioxidants directly into meat and meat products or the coating of packaging materials with plant extracts to improve their oxidative stability. The processing and storage of meat, especially industrial meat products, presents serious problems. Unsaturated lipids, fine grinding, the incorporation of air, haem pigments, contact with metals and high temperatures during processing all contribute to lipid oxidation. After microbial deterioration, the main process leading to loss of quality is lipid oxidation (Gray et al., 1996). Plant extracts have been used by several authors as natural antioxidants in meat and meat products (Table 5). The effectiveness of GSE as a food ingredient has been tested in various systems, including sunflower oil, fish oil, fish, seaweed oil emulsion, turkey, chicken, beef, pork and fish meats (Ahn et al., 2002, 2007; Lau and King, 2003; Hu et al., 2004; Pazos et al., 2005; Mielnik et al., 2006; Rababah et al., 2006; Shaker, 2006; Bañon et al., 2007; Brannan and Mah, 2007; Brannan, 2009). There is also abundant evidence demonstrating the ability of these extracts to delay lipid oxidation in meat during storage. In raw meats, GSE have been shown to be effective in reducing the amount of primary (hydroperoxides and hexanal) and secondary products of oxidation (thiobarbituric acid reactive substances [TBARS]), chicken meat (Lau and King, 2003; Shirahigue et al., 2011), pork (Brannan and Mah, 2007; Carpenter et al., 2007; Rojas and Brewer, 2008; Lorenzo et al., 2014) and fish (Pazos et al., 2005; Sánchez-Alonso and Borderías, 2008; Sánchez-Alonso et al., 2008). In cooked meats, GSE have also been shown to be effective in reducing oxidative rancidity and the formation of volatile compounds in turkey breast meat (Mielnik et al., 2006), chicken breast and thigh meat (Rababah et al., 2006; Sayago-Ayerdi et al., 2009a,b; Brannan, 2009; Selani et al., 2011), pork patties (Brannan and Mah, 2007; Rojas and Brewer, 2007, 2008; Sasse et al., 2009). These results indicate that grape polyphenols could be used as a natural ingredient to prevent oxidation and as a functional ingredient in healthy food design. A few studies published in recent years have tried to explain the mechanisms involved in antioxidant effectiveness in muscle foods. Recent data using molecular dynamic simulations with biomembranes suggests that the effects of GSE components over free radicals in fish muscle may be due to inhibition of the propagation of free radicals in the lipid bilayer, reduction in the contact of pro-oxidant compounds, such as Fe or haemoglobin, and the localization of polyphenols close to active points of oxidation (Sirk et al., 2009; Maestre et al., 2010).

Information on the transference of phenolic compounds in muscle is limited, particularly with respect to the effect of the dietary administration of polyphenols on the presence and concentration of phenolic compounds in animal tissues. Although several studies have shown that dietary polyphenols have a beneficial effect on the oxidative stability of meat, the mechanism of their action remains to be established. The direct antioxidant activity of dietary polyphenols implies that they are absorbed through the monomeric form and by the metabolites generated by the intestinal microbiota (Luciano et al., 2009). Different authors have recently shown the transmission of dietary phenolic compounds in lambs' meat (Moñino et al., 2008; Luciano et al., 2011) and frankfurters from free-range-reared pigs (Estévez et al., 2007), using rosemary and tannin-rich by-products (quebracho, grapes, nuts and citrus fruits). Moreover, Gladine et al. (2007) reported the presence of five different phenolic compounds in plasma, including epicatechin, and unknown phenolic compounds in sheep that received GS and peel extract directly into the rumen. Nevertheless, the effect of dietary procyanidins on meat oxidative stability may also be indirect, through preservation of the oxidation of other dietary bioactive compounds in the gastrointestinal tract

and in meat (for example, fatty acids and vitamins E and C), chelation of the pro-oxidant compounds, and the ability to modulate the activity and gene expression levels of relevant endogenous antioxidant enzymes (Sgorlon et al., 2006; Larrosa et al., 2010).

Part of the relationship between polyphenols and their antioxidant activity involves their transition metal-chelating potential. As dietary polyphenolic compounds show metal chelating activity (Cook et al., 1995), a high intake of these bioactive substances may have consequences for the iron status. They chelate iron by forming insoluble complexes with iron ions in the gastrointestinal lumen, thereby making the iron unavailable for absorption. Iron is also implicated in the initiation process of PUFA peroxidation (Hatcher et al., 2009). In this respect, it has been shown that the inclusion of grape polyphenols in chicken diets reduces the plasma iron concentrations associated with lower oxidation of meat (Chamorro et al., 2013). In line with this result, Marouani et al. (2007) and Lee et al. (2010) showed that the dietary addition of tea polyphenols and tannic acid significantly reduced plasma iron in rats and pigs.

The antioxidant effect of grape seed proanthocyanidins was also demonstrated by Naidoo et al. (2008) and Wang et al. (2008) in chickens infected with different dosages of *Eimeria tenella*. Results showed an increase in plasma superoxide dismutase content and a decrease in malondialdehyde (MDA) and plasma nitric oxide concentration, indicating that GSE was able to restore the balance of antioxidant–oxidant status, which had been disturbed by oxidative stress after parasite infection.

There are also references showing that the addition of grape seed by-products modifies the fatty acid composition of meat. In this respect, the dietary inclusion of a fermented GP product for pigs increased the total PUFA and PUFA/SFA ratio in the subcutaneous fat of the *Longissimus* muscle (Yan and Kim, 2011). Information on the supplemental effect of GP on the fatty acids profile of chicken meat is not available. Changes in the muscle fatty acid proportion (reduced SFA and increased PUFA) following the dietary addition of different bioflavonoids (genistein, hesperidin, gallic acid) have been reported in chickens (Kamboh and Zhu, 2013; Jung et al., 2010). Recent results from Chamorro et al. (2015) confirm that birds fed vitamin E and GP diets showed higher meat PUFA content. This effect was also correlated with a reduction in the susceptibility of the chicken meat to lipid peroxidation. Previous results indicated that monomers were better digested and that the use of tannase and pectinase released gallic acid, catechin and epicatechin (Chamorro et al., 2015; unpublished results). However, birds fed with a GP diet supplemented with enzymes (tannase) reversed the beneficial effect observed in GP diets. Although the inclusion of enzymes in GP diets hydrolysed the complex polyphenols into compounds with low polymerization, thereby improving the amount of available bioactive substances present in the gut, they are less active against oxidation and evidently no additional protective effect on oxidative stability was observed. These results suggest that simple phenols generated by the action of enzymes are less active than the more complex ones present in GP.

4.2. Antimicrobial activity and modulation of gut microbiota

In addition to its radical scavenging capacity, other valuable health-promoting activities associated with polyphenol in grape by-products have been described. The importance of dietary phenolics as antimicrobial compounds has increased in light of the growing incidence of antimicrobial resistance by certain pathogens (Table 6). The antimicrobial activity of flavonoids and other polyphenols present in different extracts of herbs and plants has been extensively documented (Smith and Mackie, 2004; Cushnie and Lamb, 2011) and reported in several recent reviews (Laparra and Sanz, 2010; Requena et al., 2010; Etxeberria et al., 2013).

There is an emerging consensus that gut microbiota may play a crucial role in the potential health benefits of polyphenols (Crozier et al., 2009). The microbiota present in the intestinal tract could metabolize dietary polyphenols into more bioactive compounds with different physiological significance and could also modify the composition and/or activity of the intestinal bacteria population (Bustos et al., 2012). Thus, dietary phenolic compounds are often transformed by gut microbiota and gut microbial population is modulated by dietary polyphenols in a two-way phenolic–microbiota interaction. Polyphenols and their derivatives affect the intestinal ecology as a significant proportion of them are not fully absorbed but are metabolized in the liver, excreted through the bile as glucuronides and accumulated in the ileal and colorectal lumen (Tzounis et al., 2008). Substantial levels of unabsorbed dietary phenolic compounds exert significant effects on the intestinal environment by suppressing or stimulating the growth of some of the components of intestinal microbiota.

It has been shown in numerous *in vitro* studies (Papadopoulou et al., 2005; Özkan et al., 2004; Rodríguez-Vaquero et al., 2007; Gañan et al., 2009; Silván et al., 2013) that flavonoids present in grape by-products have the capacity to inhibit the growth of certain organisms, such *as Staphylococcus aureus*, *Escherichia coli*, *Candida albicans and Campylobacter*. Likewise, Daroch et al. (2001) and Just and Daeschel (2003) have shown that certain polyphenolic compounds, such as resveratrol, hydroxytyrosol, quercetin and phenolic acids, also possess antimicrobial capacities against certain intestinal pathogens like *Salmonella* and *Helicobacter pylori*. Grape polyphenols also inhibit the growth of different pathogens, polymeric flavonoids (procyanidins) showing greater activity than monomeric flavonoids (Mayer et al., 2008). Cueva et al. (2010) also showed *in vitro* that microbial biotransformation of dietary phenolic compounds (phenolic acids) selectively influenced intestinal bacteria species and could affect the diversity and metabolic activity of the intestinal microbiota. Until now, only a limited number of specific bacterial species capable of dietary polyphenol degradation have been identified, for example *Eubacterium ramulus* and *Flavonifracter plautii*, previously known as *Clostridium orbiscindens* (Braune et al., 2001; Schoefer et al., 2002). In a recent study Kemperman et al. (2014) used a simulator of the intestinal microbial ecosystem to show that grape polyphenols cause changes in the gut microbial community by inhibiting *Firmicutes* and promoting *Proteobacteria*.

Table 6
Effects of grape polyphenols in *in vitro* and animal studies on gut microbiota composition.

Grape polyphenols	Study	Duration	Doses	Techniques	Antimicrobial activity	Bacteria growth promoting effect	References
Proanthocyanidin-rich GSE.	Human	2 weeks	0.5 g/day/subject extract (0.19 g/day/subject)	Counting on culture medium after faecal inoculation	*Enterobacteriaceae*	*Bifidobacterium*	Yamakoshi et al. (2001)
GSE	*In vitro* study	18–24 h incubation	Acetone:water:acetic acid (90:9.5:0.5), 667.87 mg GAE/g at 20%	Paper disc diffusion method	*A. hydrophila*, *B. cereus*, *B. brevis*		Baydar et al. (2004)
Wine polyphenols	Rat	15 weeks	0.05 g/kg	Counting on culture medium after faecal inoculation	*Clostridium* spp.	*Bifidobacterium* spp. *Bacteroides* *Lactobacillus* spp.	Dolara et al. (2005)
Resveratrol	Rat	25 days	1 mg/kg/day	Counting on culture medium after colonic inoculation		*Lactobacilli* and *Bifidobacteria*	Larrosa et al. (2009)
GSE	*In vitro* study	24–48 h incubation	GSE-Mj (414 mg/g total phenolics) at 0.25–1 mg/mL	Culture medium spectrophotometry (600 nm)	*S. thermophiles* *L. fermentum* *L. acidophilus*	*B. breve* *B. bifidus*	Tabasco et al. (2011)
GP and GSE	*In vitro* study	24 h incubation	GSE (7.2 g/kg)	Paper disc diffusion method	*C. perfringens*		Viveros et al. (2011)
	Chickens	21 days	GP (60 g/kg)	T-RFLPn	*Pseudomonas*/*Acinobacter* spp.	*Bacillus*/*Paenibacillus* spp. *Desulfitobacterium hafniense*	
			GSE (7.2 g/kg)	T-RFLPn		*Pseudomonas*/*Acinobacter* spp. – *Nocardioides* spp. – *Bacillus*/*Paenibacillus* spp. – *Desulfitobacterium hafniense*	
Wine phenolic extract	*In vitro* study	48 h	Incubation of 600 mg	Batch culture fermentation model/FISH-FCM	*C. histolyticum*		Sánchez-Patán et al., 2012
Wine polyphenols	Human	4 weeks	272 mL/day	PCR-DGGE	*Bacteroidetes* *Firmicutes*	*Fusobacteria* *Enterococcus* genus *Bifidobacterium*	Queipo-Ortuño et al. (2012)

Table 6 (*Continued*)

Grape polyphenols	Study	Duration	Doses	Techniques	Antimicrobial activity	Bacteria growth promoting effect	References
GP	*In vitro* study	22–26 h incubation	Non-Extractable polyphenols, 2.4 mg/mL	Plate count	*Bifidobacterium* spp.	*L. reuteri* *L. acidophilus* *Lactobacillus* spp.	Pozuelo et al. (2012)
	Rat	4 weeks	50 g/kg diet	RT-PCR			
GSE	*In vitro* study	24 h	50 μL	Agar plating medium	Different *Campylobacter* strains		Silván et al. (2013)
Resveratrol	Challenged pigs	4 weeks	Challenged by 2.3 × 108 cfu/ml of *E. coli* and 5.9 × 108 cfu/ml of *S. enterica*	Counting on culture medium after faecal inoculation	*E. coli* and *Salmonella*	*Lactobacillus* spp.	Ahmed et al. (2013)
GSE	Pigs	6 days	Challenged by Enterotoxigenic *E. coli* 5 ml suspension of 109 CFU/ml	Counting on culture medium after faecal inoculation		*E. coli*	Verhelst et al. (2014)
Grape seed and grape marc meal	Weaned pigs	4 weeks	10 g/kg	Counting on culture medium after faecal inoculation		*Streptoccocus* spp.	Fiesel et al. (2014)
Complex polyphenpol extracts including grape seed	Weaned pigs	3 weeks	1 g/kg	Counting on culture medium after faecal inoculation	No differences of *E. coli* or *Clostridia* counts in the faeces and caecum		Zhang et al. (2014)
GSE	Pig	6 days	10 g/kg	Counting on culture medium after faecal inoculation		*Lachnospiraceae*, *Clostridales*, *Lactobacillus* and *Ruminococcacceae*	Choy et al. (2014)
Red wine grape extract	*In vitro study*	2 weeks	1000 mg polyphenols/day	SHIME and PCR/pyrosequencing	*Bifidobacteria* *Blautia cocoides* *Anaeroglobus* *Subdoligranulum* *Bacteroides*	*Klebsiella* spp. *Alistipes* *Cloacibacillus* *Victivallis* *Akkermansia*	Kemperman et al. (2014)

GSE, grape seed extract; GP, grape pomace; GSE, grape seed extract; T-RFLPn, terminal restriction fragment length polymorphism; FISH-FCM, fluorescence *in situ* hybridization coupled to flow cytometry; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; RT-PCR, reverse transcriptase PCR.

There have been very few studies on the interaction of polyphenol compounds with intestinal microbiota in animal nutrition. *In vivo* studies have shown that resveratrol has great potential as an antibiotic alternative for reversing the adverse effects of weaning stress on the growth performance, immunity and microbial environment in *E. coli* and *Salmonella*-challenged piglets (Ahmed et al., 2013). Likewise, the addition of GSE in weaned pigs reduces *E. coli*-induced diarrhoea (Verhelst et al., 2014). Fiesel et al. (2014) showed that feeding GS and grape marc meal extract altered the microbial composition, with a reduction in *Streptococcus* spp. and *Clostridium* in the faecal microbiota. However, Zhang et al. (2014) did not observe any differences in the microbial count in the faeces and caecum of weaned pigs fed an extract containing polyphenols, including GSE. Studies conducted in rats (Dolara et al., 2005; Larrosa et al., 2009; Pozuelo et al., 2012) reported increases in the colonic populations of *Bacteroides*, *Lactobacillus* and *Bifidobacterium* that were associated with the dietary inclusion of grape seed polyphenols. An ecological shift in the microbiome, with a dramatic increase in *Lachnospiraceae*, *Clostridales*, *Lactobacillus* and *Ruminococcacceae*, was observed in female pigs fed GSE (1%) diet (Choy et al., 2014). Evidence of this effect has also been observed with the use of tea polyphenols in pigs and calves, with a significant increase in the *Lactobacilli* count, a decrease in total bacteria and *Bacteroidaceae* and a tendency towards a decrease in *Clostridium perfringens* (Hara et al., 1995; Ishihara et al., 2001). The inhibiting effect of polyphenolic compounds on bacteria could be due to mechanisms related to their capacity to adhere to cellular membranes, interact with bacterial enzymes and sequester metallic ions from the substrate (Scalbert, 1991; Cushnie and Lamb, 2011). Table 6 summarize the main results from studies analysing polyphenol-rich grape by-products and gut microbiota composition.

The results obtained by our research group on the use of GP and grape extract in birds' diets (Viveros et al., 2011) also demonstrated and confirmed the antibacterial effect of polyphenols found in these by-products with respect to certain intestinal bacteria. This effect differs depending on the segment analyzed (ileum or caecum). The inclusion of these by-products in birds' diets exerted an antimicrobial effect on *Clostridium* in the ileum, while in the caecum it was associated with an increase in populations like *Lactobacillus* and *Enterococcus*. This potential prebiotic effect was confirmed in the same study, using molecular techniques (T-RFLP) that allow global changes in microbial populations to be analyzed. These results reflected an increase in the level of biodiversity and the frequency of detection of certain bacteria with the capacity to degrade phenols, as well as other unidentified organisms in the caecum of birds fed with these by-products.

5. Conclusions and future perspectives

The beneficial effects of grape by-products are thought to derive mainly from the bioactivities of their polyphenols. However, their potential as feed ingredients or additives in animal production remains largely unexploited. These polyphenols are absorbed and metabolized by the intestinal microbiota at sufficient levels to contribute to the protection of PUFA in membranes and to modulate the antioxidant activity in intestinal content and muscle tissue. The inclusion of these by-products in feed rations enhances the oxidative stability of the meat and reduces the amount of additives required, like vitamin E. However, the optimum dose of inclusion of polyphenols in animal diets is difficult to define due to the different composition of phenolic compounds present in these by-products. Likewise, the application of antioxidants directly into meat and meat products with grape by-products improve their oxidative stability, the overall sensory and nutritional quality of meat and meat products, and hence their shelf life. Dietary polyphenol-rich grape products were also effective in increasing the growth of specific beneficial intestinal bacteria while competitively excluding certain pathogenic bacteria.

To optimize health benefits and minimize possible negative effects, more studies are needed to establish appropriate dosages of grape polyphenols and their bioavailability. It would also be necessary to characterize GP extracts and to evaluate the sensory properties and consumer acceptance of food products developed from grape polyphenols. Although, these extracts are derived from plant generally regarded as safe, but further research is needed to determine their safe limits and toxicological effects in meat and meat products as the extraction or processing conditions may alter their properties. The metabolism of polyphenols by the microbiota, the bioactivity of microbe-derived metabolites and their presence in different organs all need to be investigated. Advances in knowledge of the interaction between these bioactive compounds and the intestinal microbiota should also be a subject of increasing interest. The identification of polyphenol-metabolizing bacteria and their possible use as a probiotic could be a good strategy for increasing the bioavailability and potential bioactivity of these compounds.

Finally, the functional properties of these polyphenols could be usefully applied to animal nutrition and meat industry to meet consumer demands for healthier meat products. The inclusion of by-products from the wine-making industry in animal feed could also help to reduce the environmental and economic impact associated with their storage, transformation and disposal.

Conflict of interest

The authors declare that they have no conflict of interest.

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