

Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability

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Oxidative rancidity represents one of the major causes of deterioration in food for human consumption. Besides producing unpleasant odours, it is responsible for losses in flavour, texture, consistency, appearance and nutritional value. In a similar way, in living animals, oxidative stress constitutes an important mechanism that leads to biological damage, and is regarded as one of the causes of several pathologies that affect poultry growth. Therefore a better understanding of lipid and protein oxidation processes will allow the use of antioxidants to handle and control them. This is fundamental in order to guarantee the quality and safety of meat for human consumption, and in turn the prevention and/or delay of several oxidation processes would allow its management for an optimal quality and shelf life conservation.

Keywords: poultry; antioxidants; oxidative stress; oxidative rancidity

Introduction

Oxidative stress (OS) constitutes an important mechanism of biological damage in live animals and it is regarded as the cause of several pathologies that affect poultry growth (Dam and Glavind 1938; 1940; 1942; Goldstein and Scott 1956; Noguchi *et al.*, 1973; Van Vleet and Ferrans 1976; Perkins *et al.*, 1980; Fuhrmann and Sallmann 1995; Mezes *et al.*, 1997; Avanzo *et al.*, 2001). In a similar way, oxidative rancidity (OR) represents one of the main causes of meat deterioration (Pearson *et al.*, 1977; Sheehy *et al.*, 1993; De Winne and Dirinck, 1996; Morrissey *et al.*, 1997). Besides producing unpleasant odours, it is responsible for the loss in flavour, texture, consistency, appearance and nutritional value of the meat (Gray *et al.*, 1996; Valenzuela and Nieto, 1996).

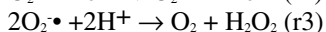
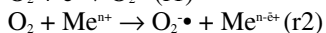
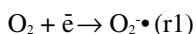
In the making of poultry diets, both fishmeal and oil can be used as a source of protein and energy. However, lipids that are high in poly-unsaturated fatty acids (PUFA) in these ingredients, specifically the ω-3, enhance the susceptibility of chicken meat to OR (Manilla and Husveth, 1999). This undesirable side effect could be reverted by adding antioxidants to the diet (Valenzuela and Nieto, 1996; Morrisey *et al.*, 1997).

Due to the importance of these processes for the oxidative stability of chicken meat, in the present review we will present concepts such as: generation of reactive oxygen species (ROS) and free radicals (FR), tissues susceptible to oxidation, antioxidant control mechanisms and topics related to broiler meat protection by adding antioxidants to the diet, in order to improve the meat oxidative stability.

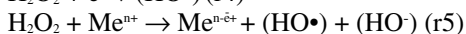
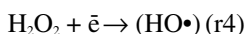
Free radicals and reactive oxygen species generation

During the processes of OR and OS oxidation products and intermediates are produced and stored. In the first case the oxidative damage affects the lipids present in abiotic systems (food), while in the second, lipids, proteins and nucleic acids present in live organisms are affected. The damage generally starts by the action of ROS, like the FR (species presenting great biological reactivity as result of one or more, unpaired electron in the most external -atomic or molecular - orbital), and those species that even not being radicals are able to promote the oxidation of susceptible substrates (pro-oxidants). The species superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical (HO^{\cdot}), derived from oxygen, and nitric oxide (NO^{\cdot}), derived from nitrogen, are the main FR present in live systems, and consequently they are involved in the OS they suffer. In OR, the non-radical singlet oxygen (1O_2) is also a promoter of oxidative processes, along with HO^{\cdot} radicals. The most external \bar{e} of singlet oxygen is in a high excitation state, having for this reason more biological reactivity.

Under normal conditions, live systems continuously generate $O_2^{\cdot-}$ radicals by monovalent reduction of molecular oxygen (reaction 1), mainly in reactions related to mitochondrial \bar{e} transport (Esterbauer, 1993). These FR can also be generated in biotic and abiotic systems by the interaction of the O_2 molecule with trace concentrations of redox-active transition metals (Me^{n+}) like Fe^{2+} and Cu^{1+} (reaction 2). Despite the low reactivity of $O_2^{\cdot-}$, this species undergoes a fast dismutation (in non enzymatic reactions as well as those catalyzed by superoxide dismutase; SOD), leading to the production of hydrogen peroxide (H_2O_2) (reaction 3).

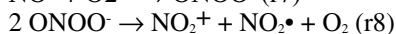
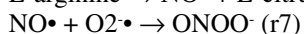
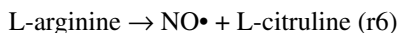


Although H_2O_2 is not a FR (since it doesn't have unpaired \bar{e}) it is an important generator of hydroxyl species (HO^{\cdot}) (reaction 4). The latter is considered to be one of the most reactive radical species to biological substratum (Halliwell, 1991; Keher, 1993). The monoreduction of H_2O_2 is a non enzymatic reaction, and like the ones with O_2 , it is easily catalyzed by redox active metals (reaction 5 – Fenton reaction).



The FR generation in live systems can be intentional and massive. An example is the ROS generation by activated neutrophils as a mechanism of organism's protection against

viruses and bacteria. Besides superoxide and hydroxyl radicals, these cells generate hypochlorous acid (HClO), a strong biological oxidant produced from chloride ions and hydrogen peroxide in a reaction catalyzed by the myeloperoxidase enzyme (Winterbourn and Kettle, 2004). Finally, the radical NO• formed from the amino acid arginine (reaction 6), is the second most abundant radical species produced in live systems, after O₂• (Halliwell, 1992). Even though NO• has a low reactivity, when it comes in contact with the O₂•, it generates peroxynitrite (ONOO⁻), a strong biological oxidant that is easily decomposed into nitronium ion (NO₂⁺, non FR) and in nitric dioxide (NO₂•, FR). This species has a high biological reactivity and behaves like HO• (Pryor and Squadrito, 1995).



Oxidative damage to susceptible substrates

Lipids, especially phospholipids present in cell membranes, are particularly susceptible to oxidative damage, being positively correlated with the degree of unsaturation of its fatty acids. In the case of FR, the attack begins by the removal of an H atom (generally adjacent to a double bond of a PUFA), leading to a lipid radical (L•). On the other hand, the peroxidative action of ¹O₂ is started by its addition to a double bond of a fatty acid, leading to a lipoperoxyl radical (LOO•). During the lipoperoxidative process started by a FR (Figure 1), in O₂ presence the radical L• generates FR LOO•. The latter is able to remove a new atom of H from an adjacent fatty acid. As a result, LOO• loses its radical character, becoming a lipohydroperoxide (LOOH) and generating a new radical L•. This process, known as lipoperoxidation in live systems and as oxidative rancidity in food, represents an oxidative chain reaction, which in the absence of antioxidants (AOX) becomes autopropagative, leading to the production of LOOH (Speisky and Jiménez, 2000). These LOOH are easily decomposed into aldehydes, ketones, alcohols, and lactones, with some of these being potentially cytotoxic to live systems (Esterbauer, 1993), and if accumulating in poultry meat they can affect its organoleptic characteristics (Pearson *et al.*, 1983; Higgins *et al.*, 1999; Ruíz *et al.*, 2001).

It is important to point out the role that haemoglobin can perform in the beginning of lipoperoxidative process. In the case of meat, it has been found that the haeme group (contains iron, Fe) present in some proteins would have an important catalytic effect in the oxidative decomposition of PUFA. The previous issue takes importance due to the fact that poultry leg and breast contain significant concentrations of haemoglobin, 0.67 mg/g and 0.24 mg/g, respectively (Kranen *et al.*, 1999) and in consequence, when the animals are slaughtered, the biochemical processes that turn the muscle into meat allow haemoproteins to control the lipoperoxidative processes that definitively accelerate the deterioration of the meat (Johns *et al.*, 1989; Andersen and Skibsted, 1991; Alayash *et al.*, 2001).

From an experimental point of view, the extent of lipoperoxidation can be evaluated by assessing the initial presence of LOOH (basal level) in samples of animal tissue or in a food, by the susceptibility of the above mentioned to undergo lipoperoxidation under prooxidizing time and/or metal-dependent conditions (both, generally reflected by the formation and accumulation of thiobarbituric acid reactive substances under experimental exposed conditions; TBARS). The TBARS test has been criticized for its lack of specificity (since it gives a positive reaction with sugars, among others), however, it has the advantage of being a simple procedure, it is easy to use and useful in the

prediction of lipoperoxidation *in vitro*. Although the TBARS assay has a good degree of correlation with other methodologies that measure lipoperoxidation (Yang *et al.*, 1991), different assays must be applied simultaneously in order to obtain more comprehensive and reliable information on the oxidative status of a sample (Del Rio *et al.*, 2002).

Proteins are a second substrate susceptible to oxidation. In this case the damage is mainly induced by FR and affects amino acidic residues. Protein oxidation (proteoxidation; PO) is a slower and less extended process than lipid oxidation. From the mechanistic point of view, the main oxidation process includes a contained Fenton-reaction (Figure 2). A transition metal joins a specific amino acid residue, allowing the generation of HO• radicals in the presence of H₂O₂ in the metal amino acid environment. Carbonyl derivatives (CO) are an important oxidation by-product of such residues, which allows determination of the extent of oxidative damage affecting the amino acid residues (mainly lysine, arginine, proline and threonine) (Gatellier *et al.*, 2000). In the same way, the lipoperoxides, and its degradation products, can also induce modifications to susceptible amino acid residues (see Figure 2). In consequence, PO could be further increased as a result of the lipoperoxidative process and, depending on the type of structural modification induced, the attacked protein could be affected on its functionality (*i.e.* loss of enzymatic activity), and/or its nutritional and organoleptic characteristics.

Antioxidants

According to origin, antioxidants can be classified as synthetic or natural. Synthetic AOX have been widely used as food preservatives, because of their effectiveness and relatively low cost. The most used antioxidants are those derived from phenolic structures, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroxyquinone (TBHQ) and dodecyl, propyl and octyl gallate (Figure 3). All of them have an admissible daily ingest (ADI). Ethoxyquin (ETOX) is another synthetic AOX with a non-phenolic structure. In contrast to the others, its consumption by humans is not allowed, but it is only used in animal diets such as in the preservation of aviary foods (Bailey *et al.*, 1996).

On the other hand, natural AOX are generally molecules present in plant parts (*e.g.* leaves, bark, seeds and/or fruits). Among the most important natural AOX are the tocopherols (or vitamin E, liposoluble) (Figure 4) and ascorbic acid (vitamin C, hydrosoluble) (Figure 5). While the former represents an essential nutrient (it must be consumed in the diet), the latter is biosynthesized by poultry (Pardue and Thaxton, 1986). Other natural molecules with antioxidant characteristics are carotenes (*i.e.* β-carotene, lycopene, lutein, astaxanthin, zeaxanthin and canthaxanthin), flavonoids (*i.e.* catechins, epigallocatechins, quercetin, rutin and morin among others), and non-flavonic phenols (*i.e.* rosmarinol and rosmaridiphenol; boldine and its analogues) (Figure 5).

Even though AOX can protect susceptible substrates by different mechanisms, their main mode of action consists of the removal of FR initiators and propagators. Clearly, this is the case for antioxidants such as vitamins E and C, and the phenolic AOX used as food preservatives. In their interaction with FR, these AOX transfer an H atom, which stabilizes the FR, becoming themselves low reactivity FR, thereby stopping the lipoperoxidative chain.

In order to use an AOX in humans and animals, its efficacy and its innocuousness needs to be priorly established. An example is the use of rosemary leaf extracts (*Rosmarinus officinalis* L.), first for pigmentation, and latter as food preservative due to its AOX components such as carnosol, rosmarinol, isorosmanol and rosmaridiphenol (Wu *et al.*, 1982). Speisky and Cassels (1994) evaluated the AOX potential of several Chilean plants,

determining that boldo leaves (*Peumus boldus*, Mol.) contains high concentrations of AOX. There are, however, many different molecules in *Peumus boldus* acting as AOX. Aporphine structures are regarded the major AOX agents, boldine being the most abundant of these.

In live organisms, reduced glutathione (GSH; hydrosoluble tripeptide synthesised by poultry), along with vitamins C and E are responsible for lowering the OS. GSH acts by releasing a hydrogen atom (attached to its thiol cysteine) and becoming oxidized glutathione (GSSG). GSH helps to stabilize FR and acts as a co-factor of glutathione peroxidase (GSHpx), an enzyme that is responsible for transforming LOOH into easily eliminated lipoalcohols. It is important to stress that this enzyme (GSHpx) contains selenium (Se) as a prosthetic group, which makes it highly dependent to the availability of this metal. It is widely established that dietary Se deficiency can cause OS in poultry (Noguchi *et al.*, 1973; Van Fleet and Ferrans, 1976; Michiels *et al.*, 1994; Avanzo *et al.*, 2001; Bozcaya *et al.*, 2001; Surai, 2002a), but this can be avoided through diet management. In fact, in a review published by Surai (2002b), the author makes a series of important and practical recommendations to improve the status of Se in poultry. The regeneration of GSH from GSSG is catalyzed by the enzyme glutathione reductase. Another important enzyme in the AOX defence is SOD, whose presence in the cell allows a fast dismutation of O_2^- to O_2 and H_2O_2 . Two types of SOD exist in eucaryotic cells: the first one incorporates the metals Cu and Zn in its prosthetic group (Cu/Zn-SOD) and occurs mostly in the cytosol, while the second one incorporates Mn in its structure (Mn-SOD) and occurs in the mitochondria (Fridovich, 1997). Supplementation of poultry diets with copper leads to an increase in the activity of the Cu/Zn dependent SOD isoform (Ozturk-Urek *et al.* 2001). A similar result was previously reported by Aydemir *et al.*, 2000, who demonstrated that the supplementation of diets with copper result in a high Cu/Zn-SOD activity in chicken erythrocytes and plasma Cu. On the other hand, Cu deficiency produces a decrease in the activity of the Cu/Zn-SOD in erythrocytes of chickens (Bozcaya *et al.*, 2001), mice (Liochev and Fridovich, 1994) and sheep (Andrewartha and Caple, 1980). Finally, the catalase enzyme (CAT) acting in concert with SOD, transforms H_2O_2 into H_2O and O_2 (Michiels *et al.*, 1994), and as with other antioxidant enzymes, it is also affected by some components of the diet. For example, Bozcaya *et al.* (2001) reported that the activity of CAT in chicken erythrocytes increases in birds with Cu and Se deficiency. More research is needed to clearly establish how these nutrients affect CAT activity.

A review published by Surai (2002a) points out that in the last 10 years it has been established that the antioxidant system with which living organisms face oxidative processes is formed by different enzymes, vitamins and minerals, which are organized in three clearly definite levels: The first level would fit with a preventive level, in which FR production would be avoided, thanks to the SOD, GSHpx and CAT enzymes, besides the metal-binding proteins. The second level would be simultaneously preventive and “curative”, since it must prevent the damage from spreading. In this level are all those breakers of chain AOX (vitamins A, C and E, carotenoids, GSH, uric acid, etc), which prevent the lipoperoxidative chain from proliferating. The third level, covering several enzymatic systems, is absolutely “curative”, and is responsible for removing or repairing damaged molecules, so they do not damage the organism.

Effect of dietary antioxidants on oxidative stress in broilers

As dietary AOX present in or added to the diet are absorbed in the gut, they can also perform a systemic function. Supplementing the diet of animals for human consumption

(i.e. poultry, pigs) with AOX has more benefits than only securing food preservation. Although the clinical manifestation of chronic pathologies related to OS in poultry is limited by their life-span (generally defined by their growth for productive purposes), various studies have pointed out the benefits of supplementing animal diets with natural and/or synthetic AOX. Highlighting the essential properties of vitamin E in poultry diets, Van Vleet and Ferrans (1976) described the changes that occur in the cells and organelles of the skeletal muscle of poultry suffering from exudative diathesis, a disease related to a lack of vitamin E and Se, and membrane oxidative damage (Dam and Glavind, 1938; 1940; 1942; Goldstein and Scott, 1956). According to Noguchi *et al.* (1973), plasma GSHpx represents the first AOX barrier for capillary cells, as it prevents attack of LOO \cdot on the PUFA membrane. Vitamin E present in the membrane acts as a second AOX barrier, by preventing the propagation of the lipoperoxidative chain. Under Se and vitamin E deficiency, none of these antioxidant mechanisms would operate, thus allowing lipoperoxidation and its disease consequences to ensue. Avanzo *et al.* (2001) confirmed that the disease is absent in chickens fed a normal vitamin E content, and also recently reported that the breast muscle of chicken deficient in such AOX shows a greater susceptibility to undergo membrane lipoperoxidation, a lower GSH content and GSH/GSSG ratio, and a decreased GSHpx activity (Avanzo *et al.*, 2001). Encephalomalacia, another disease that affects chicks, is also related to a peroxidative dysfunction produced by vitamin E deficiency (Fuhrmann and Sallmann, 1995). The cause of this disease is the oxidative damage produced in the brain of young chickens fed vitamin E-deficient diets. Some conditions present in the brain of vitamin E-deficient chickens which make them more susceptible to develop encephalomalacia are: high PUFA content, low content of vitamin E, and a decreased activity of the AOX enzymes CAT and GSHpx. All these conditions make the brain especially susceptible to undergo FR attack (Mezes *et al.*, 1997; Surai *et al.*, 1999). Furthermore, according to Perkins *et al.* (1980), nutritional muscular dystrophy (MD), which is a degenerative disease, would be influenced by an increase in FR, peroxides, and/or hydroxylperoxide concentrations. A review by Machlin and Gordon (1962) suggested that MD is manifested when chickens are fed diets low or deficient in sulphur amino acids, and according to Hull and Scott (1976), the disease is produced by a deficiency of cysteine and vitamin E. The synthesis of GSH is limited by the physiological availability of cysteine, which is synthesized from methionine (Fernandez-Checa and Kaplowitz, 2005). Deficiency of sulphur amino acids suggests a reduction in the synthesis of GSH, thus affecting the animal's AOX status and promoting the manifestation of MD. Additionally, Murphy and Kehrer (1989) established that the breast muscles of chickens affected by this disease have a higher content of vitamin E oxidation products (tocopheryl quinones; an index of OS). However they did not find significant differences in conjugated dienes and lipofuscin contents (also indicative of OS) in lipid extracts from other tissues, suggesting that there would not be enough information to support that OS causes MD, but that the damage produced by FR would be related to collateral effects of the disease.

On the other hand, dietary supplementation with vitamin E in levels higher than those required seems to improve diverse biochemical parameters related to OS in chickens. Numerous studies have reported the positive effect of vitamin E enriched diets on the susceptibility to lipoperoxidation of plasma (Sheehy *et al.*, 1994) and tissues such as muscle (Bartov and Bornstein, 1976; 1977a; 1977b; 1981; Sheehy *et al.*, 1993; 1994; Woodall *et al.*, 1996; Maraschiello, 1999), liver (Sheehy *et al.*, 1994; Applegate and Sell, 1996; Woodall *et al.*, 1996; Surai and Sparks, 2000; Husveth *et al.*, 2000), heart (Sheehy *et al.*, 1994; Surai and Sparks, 2000) and adipose tissue (Bartov and Bornstein, 1976; 1977; 1981). *Table 1* summarizes the different publications in which the effects on the lipoperoxidation and proteoxidation in different tissues was evaluated, when natural

and/or synthetic AOX were added to the diets of the animals in different doses.

In a relatively recent study, Öztürk-Ürek *et al.* (2001) found that the supplementation of poultry diets with vitamin E or C is associated with a lower lipoperoxidation basal level. This effect was observed mainly in liver, brain and heart. Furthermore, Woodall *et al.*, (1996) evaluated the effect of dietary supplementation with α -tocopherol and carotenes: β -carotene, zeaxanthin and castaxanthin, on basal lipoperoxidation and the susceptibility to lipoperoxidation of different tissues, reporting that β -carotene, zeaxanthin and α -tocopherol decreased the susceptibility to lipoperoxidation of liver, muscle and heart homogenates. Also, Tang *et al.*, (2000) reported that dietary administration of green tea catechins reduced the lipoperoxidation in muscle (both thigh and breast), liver and heart. Additionally, Biswas and Wakita (2001) reported that, besides reducing the lipoperoxidation in chicken breast, incorporation of green tea to the diet reduced fat and cholesterol deposition in the carcass of the animal.

The benefits of diet supplementation are not limited to the use of natural AOX. In fact, Wang *et al.*, (1997) reported that the early incorporation of ETOX to the poultry diet resulted in a slight increase in GSH in the duodenum, by 10% in the third week and 16% in the seventh week (at the time of slaughter weight). A higher concentration of GSH in duodenum could suggest a better removal ability of the lipoperoxides (Aw, 2005) present in the diet, thus preventing growth reduction provoked by lipoperoxide ingestion (Cabel *et al.*, 1988; Wang *et al.*, 1977). According to Wang *et al.* (1977), adding ETOX to the diet produces a slight increase (6-7%) in the body weight after 3 weeks of treatment, but not after 7 weeks. However, this supplementation does not correct for the losses in nutritional efficiency and lower weight gain produced by the presence of peroxidised fat in the diet. The latter study agrees in part with an early work by Waldroup *et al.*, (1961), who found that dietary supplementation with ETOX produced significantly higher weight gain after four weeks in one experiment, but they observed only a slight increase in weight gain after 8 weeks. Bailey *et al.* (1996) also evaluated the effect of dietary ETOX supplementation on basal lipoperoxidation in different tissues, and found a higher level in the liver and spleen of poultry fed with a diet lacking in AOX. These authors did not find, however, any differences in feed conversion, nor in bodyweight gain.

Effect of dietary antioxidants on broiler meat oxidative rancidity

The lipid composition of broiler meat is influenced by fatty acids present in their diet. As the diet becomes richer in PUFA, there is an increase in the PUFA/saturated fatty acid balance in the carcass (Bartov and Borstein, 1977a; 1977b; Grau *et al.*, 2001), promoting lipoperoxidation susceptibility in broiler meat (Marion and Woodroof, 1966; Bartov *et al.*, 1974; Bartov and Borstein, 1976).

It has been demonstrated that the systemic effect of some AOX is not restricted to an *in vivo* effect, since it can persist in the tissues *post mortem*, protecting the PUFA present in the meat. Bartov and Borstein, (1977b) studied the relation between the unsaturation level of the diet and the effectiveness of some AOX (vitamin E, ETOX and BHT) on the oxidative stability of abdominal fat and oxidative (dark) and glycolytic (white) chicken muscle. They reported that all tested AOX had a positive effect on the oxidative stability of the abdominal fat of poultry fed with saturated or unsaturated fatty acids. In turn, in chickens fed with saturated fatty acids, the addition of antioxidants, vitamin E and ETOX to the diet reduced the basal lipoperoxidation and dark muscle susceptibility to lipoperoxidation (they did not find a significant difference for light muscle). In a later study, the same authors (Bartov and Bornstein 1981) evaluated the effect of ETOX and dietary BHT alone or in combination with vitamin E. The single effect of ETOX and BHT

reduced the OR of adipose tissue; however, no significant increase in the OR of dark muscle tissue was observed. Also, there was a significant increase in vitamin E deposition in the adipose tissue, which the authors attributed to a protective effect of synthetic AOX on dietary vitamin E, or to a lower consumption of vitamin E (sparing effect). Finally, they found that the addition of these AOX in combination with vitamin E increased the vitamin deposition in the adipose tissue (compared to the addition of vitamin E alone at the same concentration) and that it substantially decreased the parameters of OR in such tissue compared to the addition of BHT, ETOX, or vitamin E alone.

Lin *et al.* (1989) demonstrated that poultry fed with diets enriched with α -tocopherol or a mixture of BHA/BHT, showed a better oxidative stability in cooled (4°C) and frozen meat (<-18°C), in addition to a significant increase in weight gain compared to chicks fed with the control diet (without AOX).

Additionally, it has been demonstrated that natural AOX can also exert a stabilizing effect on meat. Different authors have evaluated the effect of poultry diets supplemented with α -tocopherol, showing that this AOX confers a greater protection against oxidation to broiler (De Winnie and Dirink, 1996; Lopez-Bote *et al.*, 1998; Grau *et al.*, 2001) and turkey meat (Sheldon, 1997). Moreover, Lopez-Bote *et al.* (1998) investigated the effect of adding rosemary and sage extracts and vitamin E to the broiler diet on the lipoperoxidation susceptibility of meat. The authors reported a significant decrease in the lipoperoxidation levels of the white muscle of poultry fed with the natural AOX, for different cold storage periods (up to 9 days; 4°C). Although they did not find significant differences for cooled dark meat, in the case of frozen (up to 4 months; -20°C), and cooked meat, the protecting trend was similar.

The beneficial effects of vitamin E are not restricted to lipid protection, and it has also been demonstrated that they protect proteins present in turkey meat from oxidation provoked by different oxidation methods (Gatellier *et al.*, 2000; Renerre *et al.*, 1999; Mercier *et al.*, 2001). However, there are no studies showing that vitamin E has an antioxidant effect against the proteo-oxidation of cooled broiler meat when pro-oxidant conditions are not given. Thus, more investigation is needed concerning the effect that different antioxidants might have on the oxidative processes that involve the proteins contained in the poultry meat.

The research carried out to date shows that the incorporation of AOX as additives to poultry diets, not only protects food components from oxidative processes, but also promotes *in vivo* and *post mortem* effects, possibly after their absorption in the gut and incorporation to the bird's metabolism. Apparently, the AOX that are more soluble in fat (vitamin E, BHA, BHT) would be absorbed more rapidly at intestinal level, so that a certain quantity can be found deposited in tissues (Lin *et al.*, 1989), which allows efficient oxidative control of these tissues and of the meat. In case of the AOX that have a comparatively minor liposolubility (carotenoids, polyphenols), the absorption might be slower at intestinal level, or its deposition in the fatty tissues less efficient. Up to this moment, it is known that the natural AOX such as polyphenols, flavonoids, or extracts of rosemary and sage, have an antioxidant effect in meat, but the mechanisms by which this effect takes place are mainly unknown. As discussed here, although enough information exists regarding to the AOX action of vitamin E over the OE and the OR of poultry, a greater extent of research seems to be needed concerning some of the other natural antioxidants mentioned above. The latter would allow better understanding of the metabolic processes in which they are involved and can also make their future application more efficient on a productive and commercial level.

On the other hand, synthetic AOX have an established ADI, but their innocuousness has been questioned (Ito *et al.*, 1983; 1985; 1986; Masui *et al.*, 1986; Kahl and Kappus, 1993; Schildermann *et al.*, 1993a; 1993b; Iverson, 1995), due to the possibility that at high doses

some of these agents may be exert carcinogenic and/or mutagenic effects. In the case of natural AOX, their natural character should not be taken as a guarantee of their innocuousness.

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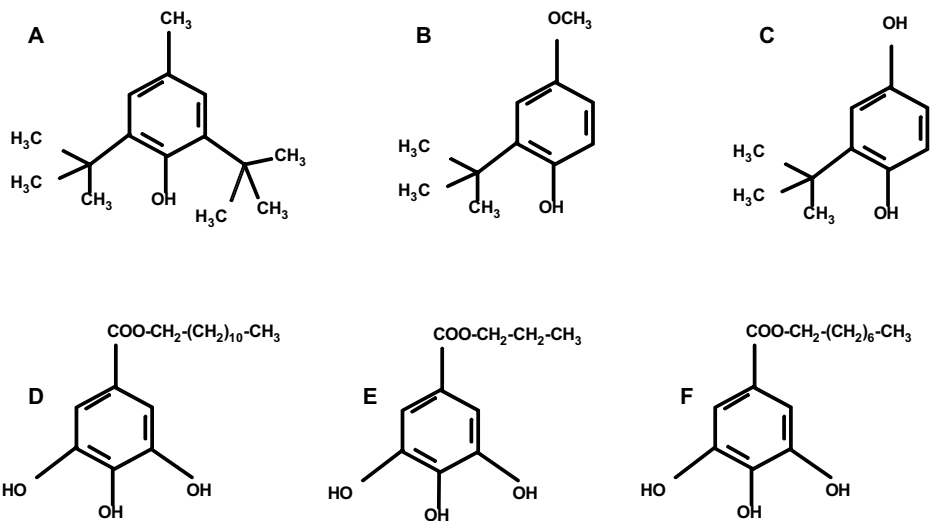


Figure 3 Synthetic antioxidants. A: BHT; B: BHA; C: t-BHQ; D: dodecyl gallate; E: propyl gallate; F: octyl gallate.

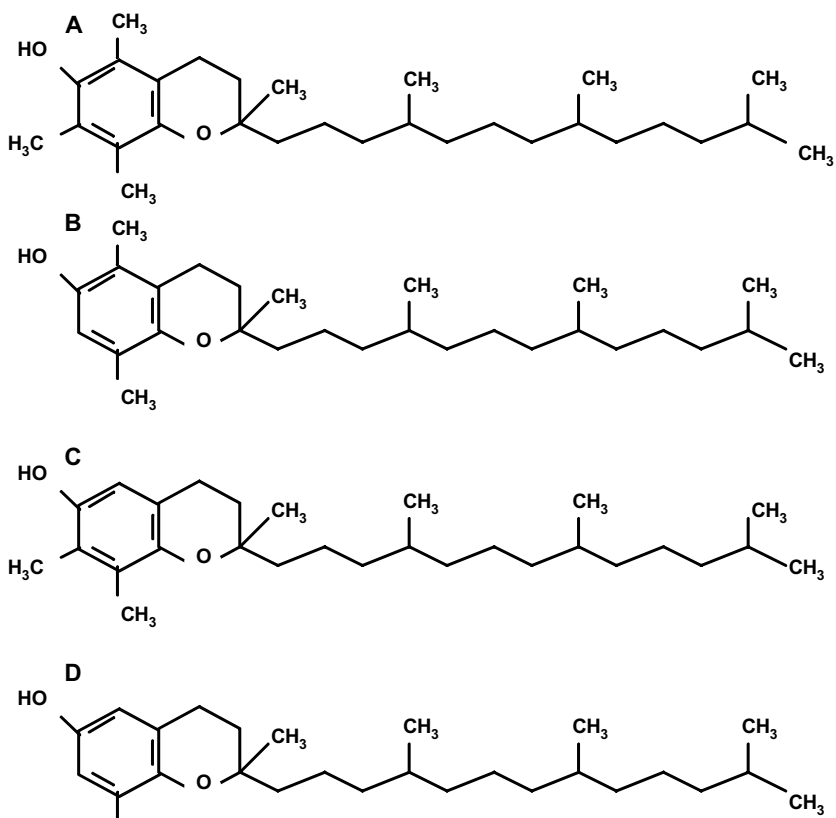


Figure 4 Natural antioxidants: Tocopherols. A: α -tocopherol; B: β -tocopherol; C: γ -tocopherol; D: δ -tocopherol.

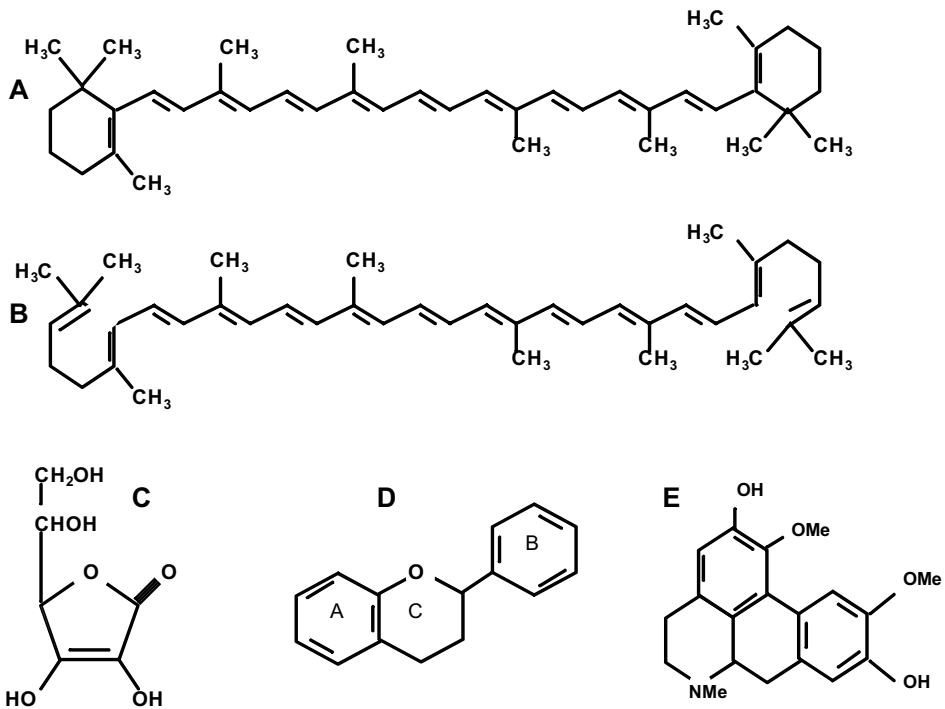


Figure 5 Natural antioxidants: A: β -carotene; B: Lycopene; C: ascorbic acid; D: Flavonoids (basic scheme); E: Boldine.

Table 1 Summary of research in which antioxidants have been applied in the nutrition of poultry.

AOX	Dose (mg/kg)	Feeding period	Effect	Reference
Ascorbic acid	110	7 to 50 days of age (slaughtered)	Pro-oxidant effect according to TBARS in broiler breast and leg after 0, 3, 5 and 7 months of freezing.	Grau <i>et al.</i> , 2001
α -tocopheryl acetate	10, 20	60 days	Reduction in lipo-peroxidation in breast and leg muscle and abdominal fat of broilers (measured according to TBARS).	Bartov and Bornstein 1977b
α -tocopheryl acetate	40	50 days	Slight content of TBARS in poultry leg meat, in comparison with the control.	Bartov and Bornstein 1981
α -tocopheryl acetate	30	50 days	Slight content of TBARS in the meat of poultry leg, in comparison with the control and major oxidative stability of the abdominal fat.	Bartov and Bornstein 1981
α -tocopheryl acetate	200	21 to 45 days of age (slaughtered)	Reduction in lipo-peroxidation breast and leg meat and abdominal fat of broilers (measured according to TBARS and sensory evaluation).	De Winne and Dirinck, 1996
α -tocopheryl acetate	200	16 weeks	Slight content of TBARS and carbonyls in the muscle of turkey, meaning less lipo-peroxidation and proteo-oxidation.	Gatellier <i>et al.</i> , 2000
α -tocopheryl acetate	225	7 to 50 days of age (slaughter)	Decrease in lipo-peroxidation in broiler breast and leg meat, raw and stewed, after 0, 3, 5 and 7 months of freezing (measured according to TBARS, to lipohydroperoxide value and to the content of cholesterol oxidation products).	Grau <i>et al.</i> , 2001
α -tocopheryl acetate	150	11 to 42 days of age	Less content of TBARS in liver, with saturated and unsaturated fatty acids in the diet.	Husveth <i>et al.</i> , 2000
α -tocopheryl acetate	200	6 weeks	Chickens had a major weight increase over the control group. The meat cooled for up to 9 days at 4°C and then frozen for up to 6 months at -20°C had a slight content of TBARS.	Lin <i>et al.</i> , 1990
α -tocopheryl acetate	200	6 weeks	Slight content of TBARS at the beginning of refrigeration for cooked leg and breast meat.	López-Bote <i>et al.</i> , 1998
α -tocopheryl acetate	200	6 weeks	Slight content of TBARS in cooked and fresh leg meat.	Maraschiello <i>et al.</i> , 1999
α -tocopheryl acetate	200	16 weeks	Decrease of the basal and induced lipo-peroxidation of turkey breast and leg meat (measured according to TBARS). Smooth decrease in the induced oxidation of breast protein (measured as carbonyls content).	Mercier <i>et al.</i> , 2001
α -tocopheryl acetate	200	1, 2, 3, 4 and 5 weeks before slaughter	The supplementation of ATA more than 1 week before slaughter caused a decrease in lipo-peroxidation susceptibility in breast, leg, heart, intestine, liver and brain tissues of broilers (measured according to TBARS). In intestine and brain tissue, 2 and 3 weeks of supplementation are enough to diminish the basal lipo-peroxidation (TBARS).	Morrissey <i>et al.</i> , 1997

AOX	Dose (mg/kg)	Feeding period	Effect	Reference
α -tocopheryl acetate	200	16 weeks	Minor TBARS content in fresh and 9 days refrigerated turkey meat.	Renner <i>et al.</i> , 1999
α -tocopheryl acetate	200	6 weeks	Differences in the rancidity were not perceived according to sensory evaluation.	Ruiz <i>et al.</i> , 2001
α -tocopheryl acetate	50	6 weeks	Oxidative stability increased of poultry meat, when chickens were fed with warmed (oxidized) oils.	Sheehy <i>et al.</i> , 1993
α -tocopheryl acetate	180	18 weeks	Less TBARS content in turkey refrigerated breast meat.	Sheldon <i>et al.</i> , 1997
α -tocopheryl acetate	300	15 to 18 weeks of age	Less TBARS content in turkey refrigerated breast meat.	Sheldon <i>et al.</i> , 1997
α -tocopheryl acetate	100		Minor TBARS content in liver and muscle.	Woodall <i>et al.</i> , 1996
β -carotene	15	6 weeks	Antioxidant effect in fresh and cooked poultry leg meat (measured according to TBARS).	Ruiz <i>et al.</i> , 1999
β -carotene	15	6 weeks	Differences in the rancidity were not perceived according to sensory evaluation.	Ruiz <i>et al.</i> , 2001
β -carotene, zeaxanthin	100		Less TBARS content in liver.	Woodall <i>et al.</i> , 1996
BHA/BHT	12.5 mg/chick/day / 12.5mg/chick/day	From the 3rd week (last 4 weeks of life) / The last 5 days before slaughter	Chickens had a major weight increase over the control group. The meat cooled for up to 9 days at 4°C and then frozen for up to 6 months at -20°C had a slight content of TBARS.	Lin <i>et al.</i> , 1989
BHT	75	60 days	Oxidative stability improvement in poultry abdominal fat.	Bartov and Bornstein 1977b
BHT	150	60 days	Improved oxidative stability of poultry abdominal fat, when the diet had a high proportion of unsaturated fatty acids.	Bartov and Bornstein 1977b
BHT	125	60 days	Oxidative stability improvement in poultry abdominal fat.	Bartov and Bornstein 1981
Tea Catechins	100, 200, 300	6 weeks before slaughter	Decrease in iron (Fe) induced lipo-peroxidation in broiler meat, liver and heart (measured according to TBARS).	Tang <i>et al.</i> , 2000
Ethoxyquin	500	6 weeks	Decreased lipo-peroxidation (TBARS content in liver and spleen).	Bailey <i>et al.</i> , 1996
Ethoxyquin	125	60 days	Improved oxidative stability of poultry abdominal fat, when the diet has a high proportion of unsaturated fatty acids.	Bartov and Bornstein 1977b
Ethoxyquin	125	50 days	Major oxidative stability in poultry abdominal fat.	Bartov and Bornstein 1981
Ethoxyquin	250	50 days	Major oxidative stability in poultry abdominal fat and leg muscle.	Bartov and Bornstein 1981
Ethoxyquin	125	7 weeks	A major growth rate increase was observed in the initial period. Increased GSH's content in intestinal tissue.	Wang <i>et al.</i> , 1997

AOX	Dose (mg/kg)	Feeding period	Effect	Reference
Ethoxyquin / α -tocopheryl acetate	125 / 40	50 days	Slight TBARS content in leg meat, compared with control. Major abdominal fat stability.	Bartov and Bornstein 1981
Rosemary extract	500	6 weeks	Slight TBARS content at the beginning of refrigeration in cooked leg meat and from the second day of refrigeration in cooked breast meat.	López-Bote <i>et al.</i> , 1998
Sage extract	500	6 weeks	Slight TBARS content at the beginning of refrigeration in cooked leg meat and from the second day of refrigeration in cooked breast meat.	López-Bote <i>et al.</i> , 1998
Green tea powder (phenolic compounds)	5000 to 15000	18 to 52 days of age	Slight TBARS content in meat, compared with control.	Biswas and Wakita, 2001
Dry tomato pulp (281 mg/kg of lycopene y 24.3 mg/kg of β -carotene)	5% / 10%	6 weeks	Have an antioxidant effect by decreasing TBARS content / Have a pro-oxidative effect, increases the TBARS content and decreases the α -tocopheryl content in breast meat	Botsoglou <i>et al.</i> , 2004