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REVIEW ON DIETARY TOCOPHEROL ACCUMULATION ON PORK TISSUES AND ITS MEMBRANE ANTIOXIDANT ROLE AGAINST LIPID OXIDATION

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Abstract

Lipid oxidation prejudices meat quality by degrading polyunsaturated fatty acids to volatile short chain compounds that are the source of undesirable flavours and odours which reduce the appeal of products to consumers.
The current review is a comprehensive study dealing with the effect the way tocopherol is absorbed by direct incorporation into diet in the intestine and then transported to the liver and distributed throughout the body, reaching out the highest amount in microsomes and mitochondria. Cumulative results of previous studies and experiments indicates that the susceptibility of meat to lipid oxidation is influenced by the α-tocopherol content of meat and PUFA content of the membrane phospholipids. Within the frame of the review we provide confirmation of the positive effect of dietary vitamin E levels on lipid oxidation by studying and analyzing published experiments where lipid oxidation was measured and where vitamin E accumulation was evaluated, established a quantitative relationship between dietary vitamin E alone and in conjunction with other antioxidants and its effects on pork meat quality.

***Key words**: vitamin E, tocopherol, meat, quality, oxidation.*

IMPLICATIONS

Changes of the national and international consumer behavior have brought new challenges for pig production.
This is not only because new legislations are continuously established, but also because the consumers are becoming increasingly conscious.
Nowadays, more consumers are concerned about the wellbeing aspects and ask for health promoting produced meat and meat products. The review is defining the perspective of using vitamin E as an antioxidant ensuring in this way the required dosage which will endow the producers with the appropriate know how to use those antioxidants to meet consumer expectations in term of pork meat quality.

INTRODUCTION

Quality (Latin for character, nature, property) is the measurable entirety of character traits, nature or quality grade of a product or service. Thus, quality traits distinguish products from other products either to their merits or deficiencies. The term meat quality concerns both the meat as a product (product quality) and the way the meat was produced (production quality) (Heyer et al., 2004). Pig meat quality is determined by many aspects, among which odour and taste are the most important attributes. Further, product quality can be divided into technological, nutritional, hygienic and sensory quality. Measurement of each of these four meat quality traits and the production quality demand different assessments and are also of varying importance for the meat purchaser. The meat processing industry might be most concerned with technological meat quality, whereas the consumer is concerned with sensory and healthy meat quality as well as production quality (Lundström et al., 2009).
A reasonable answer to these demands required by the market trend refers to a type food that contains added, technologically developed ingredients with a specific health benefit (Niva, 2007; Bhat and H. Bhat, 2011). These are food-type products that contain significant levels of biologically active components that influence specific physiological functions in the body, thereby providing benefits to health, well-being

or performance, beyond regular nutrition and are marketed and consumed for this value added property (Bhat and H. Bhat, 2011). The food industry is adopting various methods designed to maintain the quality, among those they include the use of antioxidants (Ghidurus and Varga, 2017). A multitude of studies have shown that dietary antioxidants can alleviate oxidative stress in livestock and improve the quality of animal products (Salami et al., 2016). Therefore antioxidant vitamins are essential in the animal diet for normal health (Fiego et al., 2004).

Previous studies shows that slaughter randament, the chemical composition of meat and the blood content in total cholesterol were not influenced by the type of fat introduced into the compound feed (Marin et al., 2015). The oxidative stability of muscle depends upon the balance between antioxidants, such as α-tocopherol and prooxidants including the free iron in the muscle and concentrations of polyunsaturated fatty acids (PUFA) (Yang et al., 2002). Vitamin E plays a fundamental role in the prevention of radical formation in biological systems like plasma, membranes and tissues. Vitamin E is the collective name for the eight naturally occurring forms α, β, γ and δ-tocopherols and α β, γ and δ-tocotrienols. Historically, α-tocopherol was reported to have the highest biological activity (Niculita et al., 2007). α-tocopherol and its derivatives are lipid-soluble antioxidants that suppress peroxidation of membrane lipids (Chow, 1991), it protects cells from oxygen radicals *in vivo* (Miller and Brzezinska-Slebodzinska, 1993) and *in vitro*, and is believed to be the primary free radical scavenger in mammalian cell membranes (Saikhun et al., 2008).

Swine nutritional aspects: dosage and duration of administration of tocopherol in pigs

Regarding the period of administration of the dietary supplement it was observed that the tocopherol was significantly higher in the muscles of pigs fed supplement of 200 mg α-tocopheryl acetate/kg feed for a period of 126 days before slaughter compared with pigs that were administered a low concentration of 20 mg/kg (α tocopheryl acetate/kg feed) for 91 days (Morrissey, 1996). Niculita et al. (2007) concluded that supplementation with vitamin E augment α-tocopherol levels in blood serum and muscles from pig samples receiving 300 mg/kg feed. Onibi et al. (2000) suggested that pig tissues become saturated with α-tocopherol at supplemented α-tocopheryl acetate concentrations of 200 to 500 mg/kg of diet, and α-tocopherol is no longer proportional to the dietary supply at greater concentrations.

The accumulation of α tocopherol does not reach the limits of saturation in muscle of pigs fed with 700 mg/kg α-tocopheryl acetate (Jensen et al., 1997).

In conclusion, vitamin E concentration in muscle is directly proportional to the concentration administered via animal feed and the period during which the supplement is administered vitamin E. Minimum nutritional requirements have been set in growing-finishing pigs at 11 IU/kg for vitamin E (Olivares et al., 2009; National Research Council (NRC), 1998).

Absorption and distribution of vitamin E in the tissues

The composition of biological tissues can be modified through diet, either by direct incorporation of the absorbed compounds or by their interactions with anabolic and catabolic pathways (Tres et al., 2009). Absorption of natural or synthetic form of vitamin E occurs in the small intestine. Tocopherol acetate is transformed into a tocopherol in the intestine before or during absorption. The largest portion of the tocopherol ester is hydrolyzed by esterases in the intestinal mucosa and the rest by duodenal or intraluminal esterases (Pinelli-Saavedra, 2003). Dietary tocopherol absorption is done mainly through the lymphatic system where they are transported to the chylomicrons rich in triglycerides and from here transferred to the liver. Thus vitamin E is then secreted by the liver, incorporated into very low density lipoproteins (VLDL) and finally transported to the low density lipoprotein (LDL) after previously recognized by specific LDL receptors from plasma (Herrera and Barbas, 2001). Vitamin E is also found in red blood cells and platelet cells and thus distributed throughout the body, but the highest amount of vitamin E is found in microsomes and mitochondria, which are membrane-bound organelles (Lodge, 2005).

Tocopherol concentrations in plasma and tissues are proportional to the logarithm of the amount given in the diet. Studies in mice and pigs have demonstrated that, in general, liver, skeletal muscle and fat tissue increased proportionally with increasing the dose of supplement administered from 16 to 421 mg/kg feed (Jensen et al., 1988; Niculita et al., 2007). It has been found experimentally that the level of α-tocopherol in tissues of pigs was influenced by the amount of tocopherol acetate administered in the diet of animals. Amazan et al., 2014, studied the influence of vitamin E supplementation source (natural form in water *versus* the synthetic form in feed) and dose administrated to piglets and/or sows on serum α-tocopherol concentration and antioxidant capacity on weaned piglets. He found that oral supplementation to sow with natural vitamin E as d α-tocopherol at the lowest dose produced similar concentration of α-tocopherol in serum at days 2, 4 and 28 *postpartum* to those supplemented with threefold higher dose of the synthetic form in feed. After 39 days of age neither piglet supplementation source nor dose affected α-tocopherol accumulation in the serum, muscle subcutaneous fat or liver. Asghar et al. (1991a) have quantified vitamin E isomers in the tissues of pigs fed a daily diet of all-rac tocopherol acetate. It has been found that only α-tocopherol was present in the muscle, adipose tissues and in subcellular fractions of the muscle. γ and δ isomers of vitamin E were not detectable. At subcellular level the largest amount of α-tocopherol was found in mitochondria, followed by microsomes. α-tocopherol accumulation in these cellular organelles increased linearly with level of vitamin E supplemented in swine diet. α-tocopherol accumulation in various types of tissues varies as follows: kidney tissue<lung tissue<heart tissue<liver (Monahan et al., 1994; Asghar et al., 1991a). Moreover α-tocopherol content is absorbed significantly different in the mitochondria of white muscles compared to red muscles. Trefan et al. (2011) found after a number of standardization procedures, a nonlinear relationship between the supplementary vitamin E and the accumulation of α-tocopherol in pork which approached a maximum value of 6.4 μg/g tissues. The amount of vitamin E accumulated in the muscle depends on the type of muscle fibers, their distribution, as well as their metabolic activity. O'Sullivan et al. (1997) observed that the thoracic muscles had the highest amount of vitamin E absorbed followed by cervical musculature, pelvis and shoulders.

However, *longissimus dorsi* muscles are the most studied when aiming to establish a relationship between the levels of vitamin E administrated into diet and vitamin E concentration in the muscle. This is because tocopheryl acetate will trigger an increase of α tocopherol in *longissimus dorsi* muscles of the swine (Trefan et al., 2011). Asghar et al. (1991b) analyzed the quantity of tocopherol in *longissimus dorsi* muscles and found that this muscle had the highest content of α-tocopherol. Although we have already substantial information on the action, effects and metabolism of vitamin E, there are still several questions open. The most intriguing is its interaction with other antioxidants that may explain how foods containing small amounts of vitamin E provide greater benefits than larger doses of vitamin E alone (Herrera and Barbas, 2001). An interesting finding was reported by Ching et al. (2002), regarding the accumulation of vitamin E when administrated along with vitamin A.

The result of the experiment shows that dietary vitamin E sources had no effect on serum or liver retinol concentrations. These results demonstrated that both supplemental vitamin A and vitamin E increased in the blood as their dietary levels increased. However, as dietary vitamin A level increased, serum and liver α-tocopherol concentrations declined, suggesting a reduced absorption and retention of α-tocopherol when weaned pigs were fed high dietary vitamin A levels. Olivares et al. (2009) found that in fattening pigs, feeding very high levels of vitamin A (such as 100 000 IU/kg diet) negatively affects α-tocopherol concentration in pig tissues and increases susceptibility of muscle tissue to oxidation. Muscle, adipose tissue and liver α-tocopherol concentration is not affected by dVitA in the range 1300 to 13 000 IU/kg, but liver α-tocopherol concentration is higher if vitamin A is removed from the vitamin mix 5 weeks prior to slaughter.

This suggests an interesting alternative to enhancing α-tocopherol in pig tissues. Future research is needed to establish the most adequate time/dose combination of dietary vitamin A and E in order to ensure meat quality characteristics in pigs.

The role of vitamin E in the metabolic processes and its membrane antioxidant role

In the last decade, a high number of so-called novel functions of almost all forms of vitamin E have been described, including regulation of cellular signaling and gene expression. α-tocopherol appears to be most involved in gene regulation, whereas gamma-tocopherol appears to be highly effective in preventing cancer-related processes (Brigelius-Flohé, 2006). Vitamin E is a lipid-soluble antioxidant important for the maturation and function of the immune system (Nakamura and Omaye, 2009; Hedemann et al., 2011). The most important role, however, is that *in vivo* antioxidant, which protects against free radical attack fat (Hennekens, 1998). α-tocopherol is an efficient scavenger of lipid peroxyl radicals and, hence, it is able to break peroxyl chain propagation reactions. The unpaired electron of the tocopheroxyl radical thus formed tends to be delocalized rendering the radical more stable. The radical form may be converted back to α-tocopherol in redox cycle reactions involving coenzyme Q. The regeneration of alpha-tocopherol from its tocopheroxyloxyl radical greatly enhances the turnover efficiency of α-tocopherol in its role as a lipid antioxidant (Wang and Quinn, 1999).

Most of the novel functions of individual forms of vitamin E have been demonstrated *in vitro* only and require *in vivo* confirmation. The distinct bioactivities of the various vitamins are discussed, considering their metabolism and the potential functions of metabolites (Brigelius-Flohé, 2006). Among the vitamin E isomers, RRR-α-tocopherol (α-T) has the highest *in vivo* bioavailability and bioactivity tested with different protocols, and also shows the highest H-donating activity *in vitro*. In the liver tissue, it is bound by a specific transport proteinthe α-tocopherol transfer protein (α-TTP) for distribution in circulation and thus in peripheral tissues (Galli et al., 2017). This preferential binding appears to protect this form excretion and catabolism as it occurs for the other forms. In this respect, the different forms are discriminated by the liver so that only α-T is retained and distributed to the cellular membranes of tissues, whereas the other forms are rapidly metabolized and excreted with the same mechanism of long-chain fatty acids and lipophilic xenobiotics (Ambrogini et al., 2016).

α-tocopherol protects fatty highly unsaturated against peroxidation by free radicals produced by NADPH oxidase enzymes (Benzie, 1996). By using enzymatic antioxidant mechanisms body protects itself from ROS (reactive oxygen species). The antioxidant enzymes reduce the levels of lipid hydroperoxide and H_2O_2, thus they are important in the prevention of lipid peroxidation and maintaining the structure and function of cell membranes (Nimse, 2015).

Important to note is that the addition of α-tocopherol *post-mortem* meat is not as effective as when it is used as a nutritional supplement, since the added *post-mortem* α-tocopherol cannot be incorporated directly into at the membrane level where the oxidation process is carried out (Schaefer et al., 1995). However α-tocopherol preserves the integrity of muscle cell membranes, inhibiting the passage of sarcoplasmic fluid through it, as well as acting as a radical-quenching antioxidant, consequently preventing drip loss and the oxidation of membrane phospholipids during storage when added into the diet (Asghar et al., 1991b; Gray et al., 1996; Faustman et al., 1998; Nassu et al., 2011).

The antioxidant effects of vitamin E on pork meat quality

The alteration of the meat may be a consequence of lipid and pigment oxidation in the meat (Alvares et al., 2008). Oxidation in biological tissues should be avoided because some negative biological effects have been attributed to lipid oxidation products (Guardiola et al., 2002; Niki et al., 2005; Spiteller, 2006; Tres et al., 2009). In meat and food products, oxidation might lead to a reduction of the sensory and nutritional quality (Gray et al., 1996). Nevertheless this depends on multiple factors, the most noteworthy being the systems of conservation used and the level of antioxidants present. The increase in α-tocopherol concentration in meat can be achieved through vitamin E supplementation in the animals' diet at higher levels than the nutritional requirements (Alvares et al., 2008). In order for fattening pigs to reach an optimum development under common environmental conditions, it is recommended to supplement their diet with vitamin E amounts of 15-40 mg α-tocopheryl acetate/kg fodder (Albers et al., 1984). However it has been observed that nutritional supplementation with α-tocopheryl beyond nutritional limits is an effective method to improve the quality and stability of pork meat during storage. The tocopherol accumulated in the muscles is not being degraded during the technological process thus it is exercising the effect during processing also during the storage of meat and meat products (King et al., 1995; Miller et al., 1994).

Thiobarbituric acid reactive substances (TBARS) are often used as markers for the detection of odors in meat and therefore they are used in this study as markers for the detection of oxidative degradation process. The detection limit of rancid odor in refrigerated pork meat was determined by panelists is 0.50 mg (TBARS) expressed in terms of malonic aldehyde (MA)/kg (Tarladgis et al., 1960). During administration a quantity of α tocopheryl acetate, 0-10 mg/kg feed, the critical limit of 0.50 mg TBARS expressed as MA/kg was reached after 6 days of storage under refrigeration (0.50 to 2.96 mg) (Asghar et al., 1991b; Monahan et al., 1993). In this sense, the results obtained in a study conducted by Ventanas, Tejeda and Estevez (2017) regarding the induced lipid oxidation in the muscle *biceps femoris* at different times of incubation for MA content founded that the presence of high levels of α-tocopherol in Iberian pigs muscles contributed to the lower amount of MA in muscle homogenates.

The protective effect of α-tocopherol supplementation against iron-induced lipid peroxidation has been previously described in Iberian pigs (Cava et al., 2000; Daza et al., 2005).

Sensory analysis of the meat had been reported in a few experiments, but recently it has been shown that α-tocopherol may be used in the characterization of sensory pork chops and preparations of pork chilled (freshness, softness and juicy) (Dirinck et al., 1996). Exposure to light of meat samples accelerates lipid oxidation. This negative effect produced by light can be eliminated by administering vitamin E in pig feed, as nutritional supplement. Vitamin E (DL-α-tocopherol) which is primarily active as an antioxidant has been reported to protect PUFA from free radical attack *in vivo* and *post-mortem* in animal tissues (Morrissey et al., 1994; Gabryszuk et al., 2007; Mapiye et al., 2012).

It was noted that 6 days of refrigeration in the absence of light, pork chops from animals which have not been administered the vitamin E supplement TBARS reached the level of 0.42 mg equivalent MA/kg while the addition of vitamin E (α-tocopheryl ethyl 200 mg/kg feed) TBARS reached the value of 0.21 mg which represents a 50% reduction in the amount of TBARS of the first group (Buckley et al., 1995). Various studies provide the evidence regarding the fact that dietary vitamin E supplementation decreases lipid oxidation in pork (Buckley et al., 1995; Lahucky et al., 2000; Pettigrew and Esnaola, 2001; Rosenvold and Andersen, 2003; Dunshea et al., 2005; Lahucky et al., 2005; Dikeman, 2007; Trefan et al., 2011).

CONCLUSIONS

The supplementation of animal feeds with vitamin E induces an increase in the content of vitamin E in the muscles in *post-mortem* phase. The amount of vitamin E is dependent on the type of muscle, the quantity of supplement administered (dose), and by the duration of administration. An elevated level of endogenous vitamin E ensures optimum quality for pork meat and meat products. One of the major advantages is that vitamin E reduces the rate of oxidative degradation process measured as TBARS values, thus improving the quality and stability of meat and meat products during storage.

The protective effect that vitamin E exerts over oxidation in refrigerated meat and meat products is very pronounced. Different factors during processing have an impact on shelf-life, quality characteristics and organoleptic of final product (Lengkey et al., 2016). The process of lipid peroxidation does not affect fresh meat as it is distributed and consumed in a short period of time. However a longer period of storage or refrigeration failure as far as optimum temperature for storage is concerned can accelerate the oxidation process.

An important role in maintaining the quality of meat products it is the vitamin E in animal nutrition. The protective effect of vitamin E appears to successfully complete other preservation methods.

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