EFFECTS OF ANTIOXIDANTS IN FARM LIVESTOCK

Dr Stephan Gramzow and Antje Holthausen (Cuxhaven, Germany)

Introduction

The effects of antioxidants are manifold. In the past the examination of antioxidative properties was confined to an assessment of the storage stability of fats and feedingstuffs containing fat. Against the background of increasing consumer awareness of health and nutrition, antioxidants are attracting considerable interest today in connection with the quality of processed animal products, as proved by recent studies on the effect of antioxidative activities in the living organism.

The healthy body seeks to establish an equilibrium between oxidative and antioxidative processes. An increase in oxidative processes results in a condition referred to as oxidative stress. In the absence of substances with antioxidative activity this can eventually lead to tissue damage and an increased risk of infection by affecting the immune status. As a result increased mortality can occur in broiler production, for example through pulmonary hypertension syndrome (ascites) and degenerative cardiovascular disease (sudden death syndrome). Studies in cattle have demonstrated the importance of antioxidative substances during the peripartal period, ultimately confirming the significance of antioxidants at times of physiological performance peaks.

The purpose of this paper is to explain the principles of antioxidative processes in the body and to describe the consequential effects of antioxidants in farm livestock.

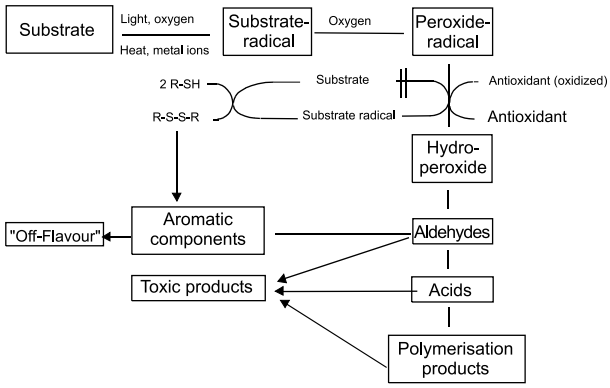
Basic concepts

First we should define what we understand by antioxidants. An antioxidant is defined by HALLIWELL et al. (1995) as any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate.

These processes, which occur automatically and are therefore referred to as autoxidation (lipid peroxidation), are free radical chain reactions and are facilitated by a number of factors. In vivo the principal factor is oxygen. It is assumed that the reduction of 25 oxygen molecules under normal conditions produces one free radical (McCORD, 1979). Free radicals are substances that possess a single unpaired electron. Given the fact that oxygen consumption can increase 10- to 15-fold and oxygen flux 100-fold during physical exertion and in stress situations (SEN, 1995), it is easy to see that the production of free radicals also rises.

Metal ions such as copper and iron also encourage the production of free radicals by their catalytic activity. An autoxidizable fatty acid molecule generates a reactive substrate radical which combines with oxygen to form a peroxide radical. The latter attacks a new, intact substrate, forming a further substrate radical. The peroxide radicals form hydroperoxides, which eventually decompose to aldehydes and ketones for example, again releasing new substrate radicals. This sets off a chain reaction. Antioxidants interrupt this chain reaction. They trap the reactive substrate radicals and peroxide radicals before they can react with oxygen or substrate. The antioxidants themselves form degradation products which are far more stable and hence slower to react than substrate radicals for example. As a result, the chain reaction is broken and the process stopped. Figure 1 illustrates the mechanism described above.

Figure 1: Schematic representation of oxidation processes (from EDER, 2001)



One of the most important antioxidants occurring in the body is vitamin E. The body cannot synthesise vitamin E itself and therefore has to rely on intake from the diet. The requirement for vitamin E depends on various factors, for example

- the body's selenium status,
- the fatty acid profile,
- the concentration of metal ions such as copper and iron,
- the amount of sulphur-containing amino acids,
- the retinol concentration.

Intakes can fluctuate heavily depending on the formulation of the ration. The vitamin E (a mixture of α-, β-, γ- und δ-tocopherols) occurring naturally in ration components (cereals, forage, natural fats and oils) is often insufficient to meet the body's needs (Table 1). The requirement of vitamin E also rises in relation to the amount and type of supplemented unsaturated fatty acids (Table 2).

Table 1: Vitamin E concentrations of different feedingstuffs (JEROCH, 1993)

Feedstuff	Content (IU/kg)
Wheat	15
Oats	12
Barley	10
Maize	30
Soya bean oil meal	2
Field beans	30
Peas	60
Soya oil	210
Rape oil	225

Table 2: Increased vitamin E requirement due to unsaturated fatty acids (MUGGLI, 1994)

Double bounds	Fatty acid		Vitamin E-requirement (IU)*
1	Oleic acid	18:1	0.13
2	Linolic acid	18:2	0.90
3	Linolen acid	18:3	1.35
4	Arachidonic acid	20:4	1.80
5	Eicosapentaenic acid	20:5	2.25
6	Docosahexaen acid	22:6	2.70

*Increased vitamin E requirement as a result of ingesting 1 g fatty acid

Moreover, free tocopherols derived from natural sources are to a large extent destroyed by oxidative processes during manufacture, storage and in the digestive tract. In order to ensure an adequate supply of vitamin E, this vitamin must be supplemented and above all adequately protected; this is achieved by using the acetate ester of α-tocopherol, the biologically most active form.

The processes which, due to the antioxidative activity of tocopherol, lead to increased consumption of vitamin E in the body are based on the oxidative production of energy in the cells, i.e. the conversion of nutrients with the aid of oxygen. Most oxygen species involved in energy production are radicals or peroxides, called pro-oxidants. While radicals and peroxides are not themselves toxic substances, their presence nevertheless poses certain dangers.

The extremely short half-lives of the oxygen radicals listed in Table 3 demonstrate how aggressive these oxygen species are.

Table 3: Reactive oxygen species and their half-lives in seconds (from PRYOR, 1986)

$^1O_2$	Singulett oxigen	10^{-6} s
H_2O_2	Hydrogen peroxide	stable
HO*	Hydroxic radical	10^{-9} s
RO*	Alcoxy radical	10^{-6} s
ROO*	Peroxic radical	7 s
ROOH	Organic hydroperoxide	stable

The body's task in this situation is to keep the radical balance in equilibrium. This function is extremely important as an excess of pro-oxidative substances destroys nutrients and micronutrients such as

- fats
- vitamin
- pigments
- amino acids and
- carbohydrates.

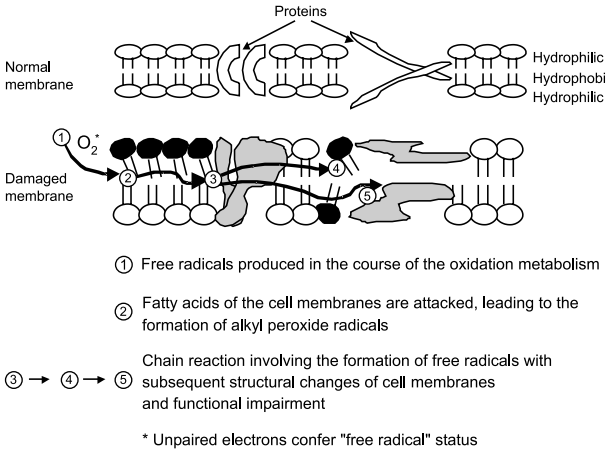
Substances involved in cell populations and tissue structures such as

- organic acids
- ribonucleotides
- proteins and
- phospholipids

are also attacked. As a result of these substances being degraded, basic biological patterns which exert functions that are vital for the body can be altered. Oxidation processes occurring in the body affect not only the protein and energy metabolism and the reactivity of the immune system, but above all the functions of cellular and mitochondrial membranes. Damage to these membranes can lead to genetic mutations by impairing cell differentiation und in the transmission of genetic information. This can ultimately result in extensive damage of the entire organism and its reproductive potential.

Figure 2 illustrates the structural and functional changes that occur in cell membranes as a result of lipid peroxidation.

Figure 2: Lipid peroxidation in the cell membrane through free radicals



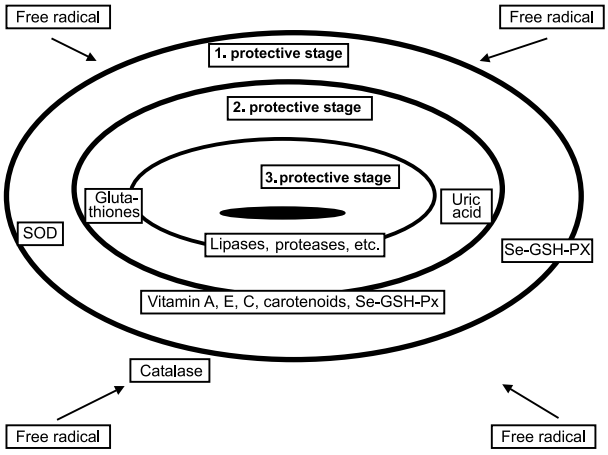
To protect themselves against cellular damage caused by peroxides and radicals formed as a result of oxidative stress, all higher organisms possess an antioxidative defence system. This defence can be divided into two mechanisms with different sites of action (Table 4).

Table 4: Antioxidative mechanisms in biological systems (after SIES, 1985)

Non-enzymatic	Vitamin E Vitamin C ß-Carotene Glutathion Flavine Organic acids Plasma proteins Technically produced antioxidants
Enzymatic	Superoxiddismutases (CuZn-, Mn-Enzymes) GSH-Peroxidases (containing selenium, free of selenium, GSH-transferases) Catalases Auxiliary enzyme (NADPH-quinon oxidoreductase) Conjugating enzymes (UDP-glucoronyl-transferase)

Figure 3 depicts three stages of antioxidative protection of cells and illustrates the interactions among these mechanisms.

Figure 3: Stages of antioxidative protection in the cell (after SURAI, 2000)



There are three principal intracellular protective stages. The first stage is based on enzymes with antioxidative activity such as superoxide dismutase (SOD) in conjunction with glutathione peroxidase (Se-GSH-Px), which contains selenium, and a catalase. This protective stage deals with the first surge of free radicals. The second stage is aimed at prevention and disruption of the chain reaction in lipid peroxidation through vitamin E for example. The central role of vitamin E as an antioxidant results from its site of action in the body. As a constituent of cell membranes it regenerates the unsaturated fatty acids of membrane phospholipids that have been attacked by radicals. If the supply of vitamin E is deficient, it is no longer available for these “repair jobs”. As a result irreparable damage is caused to the cell membranes, whose optimal function is indispensable for nutrient transport. The antioxidative activity of vitamin E continues even after slaughter. It protects the carcase and products derived from it by scavenging pro-oxidants before they can cause rancidity. The higher the vitamin E content in the cell membrane, the greater is the protective effect.

The reaction between free radicals and natural antioxidants results in the formation of hydroperoxides. These structures are toxic and have to be removed from the cell. Here vitamin E performs some of the steps needed to quench free radicals. This process is also dependent on the activity of glutathione peroxidase, which contains selenium. If concentrations of vitamin E are very high, the diet may therefore have to be supplemented with selenium so that the two stages of antioxidative cellular protection can exert their full effect.

Below we give some examples from recent studies concerning in vivo effects of antioxidants in various livestock species.

Pulmonary hypertension syndrome (PHS) - also called oedema disease or ascites syndrome - occurs primarily in broilers from three weeks of age and in laying hens; it usually involves flock outbreaks, but can also affect individual birds. The disease has also been reported in pullets and turkey poults. Economic losses through ascites syndrome are heaviest in mass outbreaks in broiler and laying hen operations. Mortality generally range from 5 to 50 % and thus represent a significant economic factor.

The pathogenesis of ascites syndrome is preferentially attributed to damage of capillary and precapillary vessels, causing escape of blood (oedematisation) and organ lesions in the heart, lungs and liver.

BOTTJE et al. (1995) studied the effect of α-tocopherol on lipid peroxidation and the incidence of ascites in growing male broilers. A control group (CNV) was kept at a ventilation rate normally used in broiler management. The broilers of three further treatments on the other hand were kept at reduced ventilation to induce the outbreak of ascites syndrome. The treatment groups were

CNV = control (normal ventilation)

CRV = control (reduced ventilation)

PL = placebo group (reduced ventilation)

VE = treatment with a vitamin E implant which released 15 mg α-tocopherol over a period from 0 to 3 weeks (reduced ventilation).

As well as recording the usual performance parameters, tissue and blood samples from diseased (PHS+) and healthy (PHS-) birds were analysed at the end of week 3 and week 5.

Figure 4 shows the liveweight development. The effect of the ventilation rate is evident. In the 1st and 2nd week broilers kept at normal ventilation were slightly lighter. The weights then equalised (week 3 and 5), whereas in week 4 higher liveweight gains were recorded at the reduced ventilation rate.

Figure 4: Liveweight of broilers during a 5-week fattening period at different dietary vitamin E levels (BOTTJE et al., 1995)

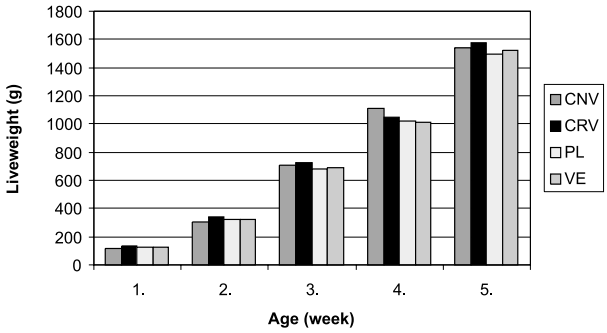


Figure 5 shows the mortality during the study period. The cumulative mortality (PHS-induced) in the test group with a vitamin E implant (VE) did not differ from the control group (CNV) and was slightly lower than in groups CR ($p<0.05$) and PL ($p=0.81$). After five weeks there were no statistically significant differences between the groups CRV, PL and VE as regards the incidence of PHS. But clinical symptoms occurred more frequencly in the experimental groups compared with the control treatment.

Plasma lipid peroxides were also raised in the PHS+ group compared with the broilers (PHS-) of the VE group and the control group. Plasma lipid peroxide levels were highest in the groups PL/PHS+ and VE/PHS+ (Figure 6).

Figure 5: Cumulative mortality (PHS-induced) of broilers at different dietary vitamin E levels (BOTTJE et al., 1995)

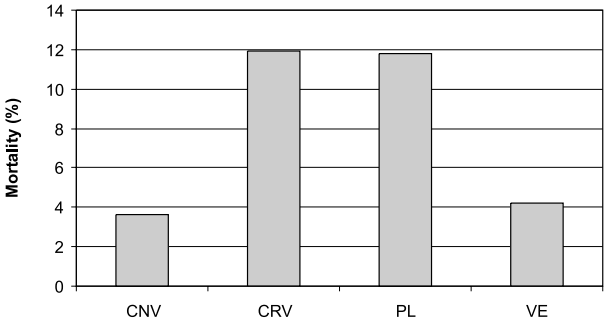
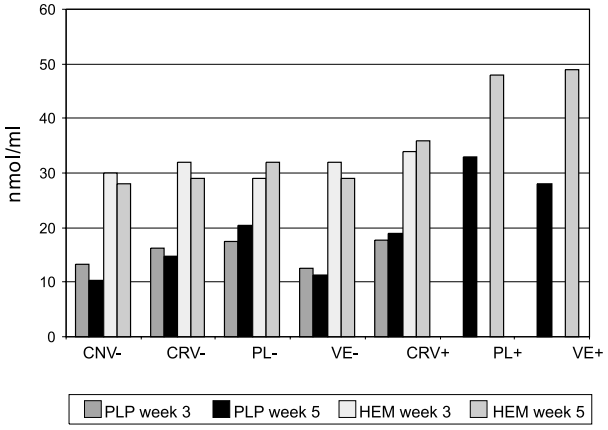
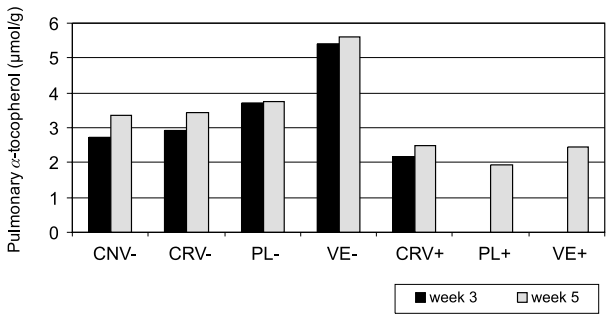


Figure 6: Plasma lipid peroxides (PLP) of broilers and hematocrit values (HEM) at different dietary vitamin E levels (BOTTJE et al., 1995)



Broilers with PHS had lower α-tocopherol concentrations in the lungs after five weeks than the unaffected birds. There were no differences between the broilers of groups CRV/PHS+, CRV/PHS- and the control group after three weeks. The pulmonary α-tocopherol concentration of group VE/PHS- was higher than in the other groups at any age, with the exception of group PL/PHS- (Figure 7).

Figure 7: Concentration of α-tocopherol in the pulmonary tissue of broilers at different dietary vitamin E levels (BOTTJE et al., 1995



The results of the studies show that a vitamin E implant (21 d) can effectively reduce the PHS-induced mortality. The lower mortality may be a result of the improved antioxidative capacity combined with a lowering of the oxidative stress induced by declining plasma lipid peroxides. The authors point out that lipid peroxidation plays a role in the development of PHS via degeneration of cardiac tissue.

BLUM et al. (1992) also observed positive effects of vitamin E supplements on mortality and immune status of broilers (Table 5).

Table 5: Vitamin E supplementation and vitamin E concentrations, immune status and mortality in broilers (BLUM et al., 1992)

Vitamin E (IU/kg)	Vitamin E (Fat tissue, mg/kg)	Mortality (%)	HI-antibody-titer (log)*
20	47	3.1	0.7
40	50	2.8	0.7
80	74	2.6	0.9
160	93	1.6	1.3

*Liveweight gain from day 7 to day 14

SOTO-SALANOVA et al. (1993) conducted studies on the effect of the dietary vitamin E concentration on the α-tocopherol status of turkey poults. The studies show that α-tocopherol levels in plama and liver tissue of turkey poults decline markedly during the first nine days after hatching. The reasons for this rapid reduction are still unclear. MECCHI et al. (1956) and MARUSICH et al. (1975) associate the low capacity for α-tocopherol accumulation in tissue early in life with inefficient intestinal absorption of vitamin E.

Antioxidants reduce the production of peroxides in the feed, thus counteracting the formation of oxidized α-tocopherol. This was essentially confirmed in the studies by SOTO-SALANOVA et al. (1993), although the effects were less pronounced than expected. Several groups were formed in the study, including

- a variant with 12 IU of DL α-tocopherol (LE),
- a variant with 12 IU of DL α-tocopherol plus 500 mg ethoxyquin/kg (LETQ),
- a variant with 100 IU of DL α-tocopherol (HE).

The age-related decline of α-tocopherol levels in the blood was again very marked. Only the HE variant showed an increase in the serum α-tocopherol concentration from nine days of age, thus counteracting this rapid reduction. The combination of a very low vitamin E concentration with an antioxidant (ethoxyquin) produced no benefits. This may be due to the distinctly lower vitamin E supply compared with the HE variant. Slightly improved liver and serum concentrations of α-tocopherol were observed in some cases (Table 6).

Technical antioxidants can simulate the antioxidative action of Vitamin E but not its physiological functions. The **site of action of technical antioxidants in vivo is the digestive tract**. Here digestive processes cause the formation of numerous free radicals which have to be deactivated by antioxidants.

Table 6: Liver and serum concentrations of α-tocopherol in turkey poults at different vitamin E doses and supplementation of ethoxyquin

	Day 1	Day 5	Day 9	Day 14	Day 21
µg α-tocopherol/ g liver					
LE	76.75	15.75	1.50	0.31	0.29
LETQ	76.75	21.48	0.91	0.38	0.35
HE	76.75	20.28	2.32	1.70	1.98
µg α-tocopherol/ ml serum					
LE	3.03	0.83	0.38	0.22	0.33
LETQ	3.03	0.97	0.40	0.25	0.30
HE	3.03	1.30	1.01	1.06	1.67

HARMS et al. (1984) conducted a trial in laying hens on this topic. The laying hens received an antioxidant strictly via the drinking water. This means that the antioxidant could only exert its effect in the gastrointestinal tract. Table 7 shows the results of this trial. The test parameter was the colour of the egg yolk, which was measured by various methods. As measured by the colour intensity of the yolks, significantly more carotenoids were deposited in the egg after administration of 2.2 mg ethoxyquin (ETQ) per ml water. This implies that fewer carotenoids were destroyed by oxidation with this treatment. The experimental design means that this effect is not attributable to any activity in the feed as the antioxidant was administered via the drinking water. The protective effect of the antioxidant administered with the drinking water on the carotenoids can therefore only have occurred after feeding in the digestive tract before absorption.

Table 7: Colour intensity and dominant wave length in the egg yolk and feed intake of laying hens relative to the ethoxyquin concentration in the drinking water (HARMS et al., 1984)

Ethoxyquin in drinking water (mg/ml)	Colour intensity (%)	Wave lenght (nm)	Feed intake (g/hen/day)
0	84.38	578.3	98.4
1.2	85.37	578.3	100.4
2.2	86.17	578.4	99.5

Free radicals are becoming an increasingly important issue in veterinary medicine. An example of this is abomasal displacement in dairy cows. Rising milk yields have led to a worldwide increase in the incidence of this condition. At a production level of 6,000 to 8,000 kg FCM/year up to 5 % of dairy cows have problems with abomasal displacement and on some farms the proportion can be considerably higher (FÜRLL et al., 1996).

It was found that repositioning the displaced abomasum did not lead to the expected improvement but instead caused a deterioration in the animals' condition. As a result of the resumption of blood flow to tissue temporarily damaged by pressure (ischaemia), even more radicals are produced, leading to reperfusion damage. These reperfusion injuries are caused by the reflux of oxygen into previously damaged tissue. The injuries take the form of lipid peroxidation, membrane damage and increased vascular permeability. As a consequence an increase in the concentration of inflammatory factors can be expected to occur (MÜLLER-PEDDINGHAUS, 1987).

FÜRLL et al. (1999) studied a potential improvement of the antioxidative status in five Holstein-Friesian cows with left abomasal displacement. The animals received either 5 g ascorbic acid intravenously or 10 mg Na-selenite in combination with 1 g α-tocopherol intramuscularly. A further five animals with left abomasal displacement received no additional medication. The results confirm the beneficial effects of the vitamin supplement and the combination with Na-selenite. Treated animals with left abomasal displacement showed a more rapid decline of fatty acid concentrations to normal levels than untreated animals. Apparently the clinical condition of the treated animals improved more rapidly than that of the control animals, as shown by a resumption of ruminal activity as early as 24 hours after repositioning of the abomasum (Table 8).

Table 8: Free fatty acids in the serum of groups of 5 Holstein-Friesian cows with left abomasal displacement before and after repositioning of the abomasum (FÜRLL et al., 1999)

	FFA (mmol/l)		
	Before surgery	h after repositioning	
		1	24
Control	0.958	1.014	0.698
Vitamin C	1.416	1.482	0.548
Vitamin E	1.386	1.250	0.516

Concluding remarks

Adverse effects of oxidative processes can be considerably reduced by supplementation of technical antioxidants, thus reducing energy and nutrient losses. In this way the amount of endogenous vitamin E available to the animal is "spared" as the oxidation process occurring in the intestinal tract is interrupted primarily by technically produced antioxidants. Recent studies confirm the positive effects of high vitamin E concentrations on the quality of products derived from farm animals as well as on mortality and immune status.

In order to utilise the varied and important functions of vitamin E effectively, rations enriched with vitamin E should additionally be supplemented with a technically produced antioxidant. This ensures that the tocopherols present in the feed only act to a limited extent as antioxidants before crossing the intestinal barrier because this function is taken over by the technical antioxidant, thus maximising the amount of vitamin E available to the body.

Literature

BLUM, J.C., M. FRIGG, F.H. RICARD, M.R. SALICHON, C. TOURAILLE (1992): Effect of dietary vitamin E supplies in broilers. 2nd report: Male and female growth rate, viability, immune response, fat content and meat flavour variations during storage. Arch. Geflügelk. 56, 37-42

BOTTJE, W., B. ENKVETCHAKUL, R. MOORE (1995): Effect of α-Tocopherol on antioxidants, lipid peroxidation, and the incidence of pulmonary hypertension syndrome (ascites) in broilers. Poultry Science 74, 1356-1369

EDER, K. (2001): personal communication

FÜRLL, M., E. SCHMIDT, L. JÄKEL, M.N. DABBAGH, U. SCHARZER, T. LEIDEL., U. MÜLLER, G. SCHNEIDER (1996): Zum Vorkommen der Dislocatio abomasi in Ostdeutschland. Tierärzt. Umschau 51, 221-215

FÜRLL, M., M.N. DABBAGH, B. FÜRLL, T. SATTLER, C. SPIELMANN (1999): Ätiologie und Prophylaxe von Reperfusionsschäden. Dtsch. tierärztl. Wochenschr. 106, 389-293

HALLIWELL, B., R. AESCHBACH, O.I. ARUOMA, J. LÖLIGER (1995): The characterization of antioxidants. Fd Chem. Toxic. Vol. 33, No. 7, 601-617

HARMS, R.W., R.E. BURESHI, B.L. DAMRON (1984): The in vivo benefit of ethoxyquin for egg yolk pigmentation. Poultry Science 63, 1659-1660

JEROCH, H., G. FLACHOWSKY, F. WEISSBACH (1993): Futtermittelkunde. Publisher Gustav Fischer, Jena und Stuttgart

MARUSICH, W.L., E. DeRITTER, E.F. OGRINZ, J.KEATING., M. MITROVIC, R.H. BUNNELL (1975): Effect of supplemental vitamin E in control of rancidity in poultry meat. Poultry Science 54, 831-844

McCORD, J.M. (1979): Superoxide, superoxide dismutase and oxygen toxicity. Reviews in biochemical toxicology. Vol. 1, 109-124

MECCHI, E.P., M.F. POOL, G.A. BEHMANB, M. HAMACHI, A.A. KLOSE (1956): The role of tocopherol content in the comparative stability of chicken and turkey fat. Poultry Science 35, 1238-1246

MUGGLI, R. (1994): Physiological requirements of vitamin E as a function of the amount and type of polyunsaturated fatty acid. World Review of Nutrition and Dietetics 75, 166-8

MÜLLER-PEDDINGHAUS, R. (1987): Pathophysiologie und Pharmakologie reaktiver Sauerstoffspezies bei der Entzündung. Arzneim.-Forsch./Drug Res. 37 (1), 589-600

PRYOR, W.A. (1986). Annu. Rev. Physiol. 48, 657

SEN, C.K. (1995): Oxidants and antioxidants in exercise. J. Appl. Physiol. 79 (3), 675-686

SIES, H. (1985): Oxidative stress. Academic Press, 18

SOTO-SALANOVA, M.F., D.L. BARKER, R.C. EWAN, F. JAVIER PIQUER, E.G. MALLARINO, P.E. PALO, J.L. SELL (1993): Research Note: Vitamin E status of turkey poults as influenced by different dietary vitamin E sources, a bile salt, and an antioxidant. Poultry Science 72, 1184-1188

SURAI, P. (2000): Organic selenium and the egg: Lessons from nature. Feed Compounder Nov. 2000, 16-18