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RECENT DEVELOPMENTS IN USAGE OF NATURAL ANTIOXIDANTS TO IMPROVE CHICKEN MEAT PRODUCTION AND QUALITY

A. A. FOTINA¹, V. I. FISININ² and P. F. SURAI³

Abstract

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For the majority of organisms on Earth, life without oxygen is impossible. Animals, plants and many microorganisms rely on oxygen for the efficient production of energy. However, the high oxygen concentration in the atmosphere is potentially toxic for living organisms. Recently free radical research has generated valuable information for further understanding not only detrimental, but also beneficial role of free radicals in cell signalling and other physiological processes. The benefit or harm of free radicals ultimately depends on the level of their production and efficiency of antioxidant defence. During evolution, living organisms have developed specific antioxidant protective mechanisms to deal with ROS. Therefore, the presence of natural antioxidants in living organisms is the major factor that enables their survival in an oxygen-rich environment. The protective antioxidant compounds are located in organelles, sub cellular compartments or the extra cellular space, enabling maximum cellular protection to occur. Natural antioxidants, including vitamin E, Se, carotenoids, carnitine and some others play important roles in chicken nutrition by maintaining antioxidant defences of their tissues. Optimal antioxidant supplementation is shown to be important to maintain high growth rate, immunocompetence and meat quality of growing broiler chicks. A new technology based on vita-gene concept has been developed to deal with various stresses by supplementing a complex mixture of antioxidants and other important elements via drinking water.

Key words: chicken, antioxidants, stress, meat quality

Introduction

Commercial broiler production is associated with various stresses decreasing productive and reproductive performance of chickens. It is proven that most of stresses in poultry production (technological: chick placement or thinning; environmental: heat stress, high ammonia, etc.; nutritional: mycotoxins, misbalances of vitamins and minerals, etc.; or internal: bacterial and viral challenges) at the cellular level are associated with oxidative stress due to access of free radical production or inadequate antioxidant protection (Surai, 2002; 2006; Surai and Fisinin, 2012). Therefore, the antioxidant system of the body is responsible for prevention damages caused by free radicals to various biological molecules including proteins, lipids and DNA. Many of natural antioxidants are

provided with the chicken diet (vitamin E, carotenoids, selenium, etc.), while a range of other antioxidant compounds are synthesised in the body (glutathione, thioredoxins, antioxidant enzymes, etc.) and a delicate balance between antioxidants and pro-oxidants in cells, digestive tract and in the whole body is responsible for maintenance of chicken health, their productive and reproductive performances.

The aim of this paper is to review roles of natural antioxidants in broiler production.

Free Radical Production

Free radicals are atoms or molecules containing one or more unpaired electrons. Free radicals are highly unstable and reactive and are capable of damaging biologically relevant

E-mail: psurai@feedfood.co.uk

¹ Sumy National Agrarian University, Sumy, Ukraine

² All Russian Institute of Poultry Husbandry, Sergiev Posad, Russia

³ Feed-Food .Ltd, UK; Scottish Agricultural College, UK; Odessa National Academy of Food Technology, Odessa, Ukraine

molecules such as DNA, proteins, lipids or carbohydrates. The animal body is under constant attack from free radicals, formed as a natural consequence of the body's normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms. Recently collective terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been introduced including not only the oxygen or nitrogen radicals, but also some non-radical reactive derivatives of oxygen and nitrogen (Surai, 2006).

Superoxide (O₂*) is the main free radical produced in biological systems during normal respiration in mitochondria and by autoxidation reactions with half-life at 37°C in the range of 1 x 10-6 second. Superoxide can inactivate some enzymes due to formation of unstable complexes with transition metals of enzyme prosthetic groups, followed by oxidative self-destruction of the active site. Depending on condition, superoxide can act as oxidizing or a reducing agent. It is necessary to mention that superoxide, by itself, is not extremely dangerous and does not rapidly cross lipid membrane bilayer. However, superoxide is a precursor of other, more powerful ROS. For example, it reacts with nitric oxide with a formation of peroxynitrite (ONOO-), a strong oxidant, which lead to formation of reactive intermediates due to spontaneous decomposition.

Hydroxyl radical is the most reactive species with an estimated half-life of only about 10⁻⁹ second. It can damage any biological molecule it touches; however, its diffusion capability is restricted to only about two molecular diameters before reacting. Therefore, in most cases damaging effect of hydroxyl radical is restricted to the site of its formation. In general, hydroxyl radical can be generated in human/animal body as a result of radiation exposure from natural sources (radon gas, cosmic radiation) and from man-made sources (electromagnetic radiation and radionuclide contamination). In fact, in many cases hydroxyl radical is a trigger of chain reaction in lipid peroxidation (Surai, 2005).

Therefore, ROS/RNS are constantly produced *in vivo* in the course of the physiological metabolism in tissues. It is generally accepted that the electron-transport chain in the mitochondria is responsible for major part of superoxide production in the body (Halliwell and Gutteridge, 1999). Mitochondrial electron transport system consumes more than 85% of all oxygen used by the cell and, because the efficiency of electron transport is not 100%, about 1-3% of electrons escape from the chain and the univalent reduction of molecular oxygen results in superoxide anion formation. About 10¹² O₂ molecules processed by each rat cell daily and if the leakage of partially reduced oxygen molecules is about 2%, this will yield about 2 x10¹⁰ molecules of ROS per cell per day. An interesting calculation has been made by Halliwell (1994), showing that in the human body about 1.72 kg/year of super-

oxide radical is produced. In stress condition it would be substantially increased. Clearly, these calculations showed that free radical production in the body is substantial and many thousand biological molecules can be easily damaged if are not protected. Recently the role of mitochondria as a permanent source of ROS has been questioned.

The most important effect of free radicals on the cellular metabolism is due to their participation in lipid peroxidation reactions. In fact, lipid peroxidation is a chain reaction and potentially large number of cycles of peroxidation could cause substantial damage to cells. In membranes, the peroxidazable material is represented by PUFAs. It is generally accepted that PUFA susceptibility to peroxidation is proportional to amount double bounds in the molecules. In fact, docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) are among major substrates of the peroxidation in the membrane. It is necessary to underline that the same PUFAs are responsible for maintenance of physiologically important membrane properties including fluidity and permeability. Therefore, as a result of lipid peroxidation within the biological membranes their structure and functions are compromised. Proteins and DNA are also important targets for ROS.

The complex structure of proteins and a variety of oxidizable functional groups of the amino acids make them susceptible to oxidative damage. In fact, the accumulation of oxidized proteins has been implicated in the aging process and in other age-related pathologies. A range of oxidized proteins and amino acids has been characterised in biological systems. In general, the accumulation of oxidized proteins depends on the balance between antioxidants, prooxidants and removal/repair mechanisms. Oxidation of proteins leads to the formation of reversible disulfide bridges. More severe protein oxidation causes a formation of chemically modified derivatives e.g. shiff's base (Surai, 2006). Nitric oxide, hydroxyl radical, alkoxyl and peroxyl radicals as well as carbon-centered radicals, hydrogen peroxide, aldehydes or other products of lipid peroxidation can attack protein molecules. Usually oxidative modification of proteins occurs by two different mechanisms: a site-specific formation of ROS via redox-active transition metals and non-metal-dependent ROS-induced oxidation of amino acids. The modification of a protein occurs by either a direct oxidation of a specific amino acid in the protein molecule or cleavage of the protein backbone. In both cases, biological activity of the modified proteins would be compromised. The degree of protein damage depends on many different factors (Surai, 2002; 2005; 2006; Surai and Fisinin, 2010):

 the nature and relative location of the oxidant or free radical source;

- nature and structure of protein;
- the proximity of ROS to protein target;
- the nature and concentrations of available antioxidants.

Normally, there is a delicate balance between the amount of free radicals generated in the body and the antioxidants to protect against them. For the majority of organisms on Earth, life without oxygen is impossible, animals, plants and many microorganisms relying on oxygen for the efficient production of energy. However, they pay a high price for pleasure of living in an oxygenated atmosphere since high oxygen concentration in the atmosphere is potentially toxic for living organisms. Formation of ROS in foods during storage, processing and cooking is closely interrelated among ROS. The most important ROS are hydroxy radical and singlet oxygen. Hydrogen peroxide and superoxide anion are important precursors for hydroxyl radical and singlet oxygen formation. It is extremely important to control the formation of ROS in foods to improve the food quality.

Antioxidant Protection

During evolution, living organisms have developed specific antioxidant protective mechanisms to deal with ROS and RNS (Surai, 2002). Therefore, it is only the presence of natural antioxidants in living organisms, which enable them to survive in an oxygen-rich environment. These mechanisms are described by the general term "antioxidant system". It is diverse and responsible for the protection of cells from the actions of free radicals. This system includes:

- Natural fat-soluble antioxidants (vitamins A, E, carotenoids, ubiquinones, etc.);
- Water-soluble antioxidants (ascorbic acid, uric acid, taurine, etc.)
- Antioxidant enzymes: glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD).
- thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase and a thioredoxin system (thioredoxin/thioredoxin peroxidase/thioredoxin reductase).

The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur. Antioxidant system of the body is responsible for prevention of damaging effects of free radicals in stress conditions. Therefore, dietary supplementation of antioxidant compounds is a way to improve efficiency of broiler production in commercial conditions associated with various stresses.

Dietary-derived antioxidants related to broiler nutrition can be divided into several groups:

 Vitamin E – main chain-breaking antioxidant in the cell, located in biological membranes and proven to be effective in antioxidant protection. Recently it has been proven that vitamin E recycling in the cell is a key for its antioxidant activity. Ascorbic acid, selenium, vitamins B1 and B2 are important elements of vitamin E recycling. Therefore, if recycling is effective even low vitamin E concentration, for example in embryonic brain, can prevent lipid peroxidation in vivo (Surai et al., 1996). Dietary vitamin E supplementation is an important part of chicken meat production technology (Surai, 1999; 2002).

- Selenium an integral part of 25 selenoproteins participating in various antioxidant reaction in the body. It is supplied with the diet in various forms and optimal Se status is a key for effective antioxidant protection (Surai, 2006; Pappas et al., 2008).
- Carotenoids important elements of antioxidant system, possessing antioxidant activities and participating directly or indirectly (for example, by recycling vitamin E or regulating expression of various genes) in antioxidant defences. There are more than 750 carotenoids in nature and their efficiency vary considerably. Recently, an important role of canthaxanthin in breeder nutrition has been described (Surai, 2012a; 2012b).
- Vitamin C (ascorbic acid) an important antioxidant synthesised in chickens and its dietary supplementation is shown to be effective only in stress conditions, when its requirement substantially increased. The role of vitamin C in vitamin E recycling is a topic of great interest.
- Polyphenolic compounds a group of various plan-derived compounds comprising more than 8,000 various compounds possessing antioxidant and pro-oxidant properties in various conditions (Surai, 2013). The main problem with polyphenols, including flavonoids, is their low bioavailability. Their concentration in the diet could be very high, but their levels in blood are low and their concentration in target tissues (liver, muscles, egg yolk) usually is negligible. Therefore, main site of flavonoid action is the gut where they can have health-promoting properties participating in the maintaining antioxidant-prooxidant balance (Surai et al., 2004; Surai, 2006).

Usage of natural antioxidants in broiler production could be divided into several stages:

- Breeder nutrition in order to prevent oxidative stress during embryonic development and in early postnatal development
- Broiler nutrition to maintain high growth and strong immunity
- Broiler nutrition to increase meat stability during processing and storage
- Addition of antioxidants during meat processing, including packaging.

Breeder Nutrition to Avoid Oxidative Stress during Embryonic Development

This topic was recently reviewed (Surai and Fisinin, 2012b; Surai and Fisinin, 2013) and can be summarized as follows:

Dietary vitamin E supplementation increases antioxidant protection of the developing embryo. Vitamin E is effectively transferred from the feed to the egg yolk and further to the embryonic liver and other tissues. The highest vitamin E concentration was found in the embryonic liver at time of hatching. It is considered to be an effective adaptive mechanism to deal with hatching stress. It was proven that increased vitamin E concentration in the diet is associated with its accumulation in the egg yolk, embryonic liver and other embryonic tissues significantly decreasing lipid peroxidation.

Carotenoid dietary supplementation is associated with their transfer to the egg yolk and embryonic liver similar to that described for vitamin E. Protective antioxidant effect of carotenoids depends on the efficiency of their assimilation from the diet. It was proven that canthaxanthin is a carotenoid of choice for improvement of antioxidant defences of the embryo and newly hatched chicks (Surai, 2012a; 2012b).

Selenium is transferred from the feed to the egg yolk and egg white. The efficiency of transfer depends on the form of selenium with organic selenium (Selenomethionine, SeYeast or other organic forms) being more effective to increase Se level in eggs and embryonic tissues. Increased Se content in chicken embryo is associated with decreased lipid peroxidation (Surai, 2006).

Vitamin C is not effective, since it is not transferred to the egg

Polyphenols, including flavonoids, are not effective since they are not transferred to the egg

Broiler Nutrition to Maintain Growth and Optimal Health

Vitamin E dietary supplementation varies depending on country and conditions of broiler production. In general, broiler production in countries with high temperature impose temperature stress and vitamin E supplementation substantially increased (100 ppm and higher), while in countries with continental climate its supplementation at 20-50 mg/kg is a commercial standard. Vitamin E possessing immunomodulating properties (Hernández et al., 2009; Konjufca et al., 2004). Many attempts to find an optimal vitamin E dose to maintain high immunocompetence did not find an ultimate answer, since immune-response to vitamin E is not linear (Boa-Amponsem et al., 2006; Siegel et al., 2006; Leschinsky and Klasing, 2001;

Friedman et al., 1998) and this field needs more research. It seems likely that benefits of natural vitamin E vs synthetic one is still not high enough to justify the difference in price.

Carotenoids are widely used for skin coloration in countries where yellow skin broilers are preferable. There is a range of carotenoids designed to have best results, including synthetic carotenoids as well as carotenoids extracted from various plants (Surai, 2002). Carotenoid supplementation to increase antioxidant defences during broiler production needs further investigation. It was shown that chickens hatched from carotenoid enriched eggs are more effectively assimilate carotenoids from the diet in postnatal development. Carotenoids possess immunomodulating properties (Blount et al., 2003) and various research groups expanding this idea with wild birds and these needs to be done with broilers as well.

Selenium is an effective means of increasing antioxidant defences during postnatal chicken development and optimal concentration and form of Se in the diet are key elements to the successful broiler growth and high immunocompetence (Surai, 2006).

Carnitine is an important element of the antioxidant system of the body regulating mitochondria function has positive effects on chickens when added with feed or supplemented via water (Golzar Adabi et al., 2011). It also has immunomodulating properties in chickens (Buyse et al., 2007; Deng et al., 2006) and needs further investigation. Indeed, carnitine is synthesized in animals, including chickens, however, in stress conditions its synthesis is most likely is not sufficient and there is a need for its supplementation. In general, carnitine supplementation via drinking water is considered as an effective means of improving carnitine status of the birds (Arslan, 2006) and their performance.

Vitamin C is an important element of antioxidant defense in stress conditions and its increased supplementation in combination with other antioxidants could be beneficial, especially during various stress conditions.

Polyphenols and various plant extracts are widely used in resent research trial in attempt to replace traditional antioxidants (vitamin E), but taking into account their low bioavailability and potential pro-oxidant effect it is too early to make commercial applications (Surai, 2013). In general, polyphenolic compounds in the broiler diet could help to maintain healthy gut, improve defense against various microbial compounds, regulate gene expression, etc. However, the main conclusion, which is clear from the existing research data, is that polyphenols cannot replace vitamin E in poultry and animal nutrition. They have their own role to play, but their antioxidant activities is probably not the main mechanism of their action on animals (Surai, 2013).

Other antioxidant compounds, which already found their way to poultry diet, are carnitine and betaine. These compounds participate in antioxidant defenses via regulation of vita-genes and need further investigations.

Broiler Nutrition to Increase Meat Stability during Processing and Storage

For the last few years consumer demands regarding aspects meat quality have substantially increased. Therefore, a challenge to the meat industry is to enhance the image of meat purchased at the supermarket (Janssens, 1998). Many meat quality characteristics attract consumer attention. They include appearance, texture and flavour (Liu et al., 1995) as well as tenderness, juiciness, aroma (Janssens, 1998) and other subjective characteristics. Among this appearance has a major impact on the initial decision of the customer to purchase or reject the product (Sheehy et al., 1997). Consumers prefer fresh meat with a minimum loss of water during handling and cooking. Therefore, water-holding capacity of the meat (Mahan and Kim, 1999) as well as colour (Froning, 1995) and absence off-flavours (Sheehy et al., 1997) are considered among most important meat quality characteristics. It has been shown that sensory quality of meat is affected by muscle biochemistry and modern processing technologies (Ouali, 1991). For example, grinding increases oxygen incorporation into muscle and cooking releases protein-bound iron into the intracellular pool (Chan and Decker, 1994). As shown above, in this process free radical production and lipid peroxidation cause membrane structure disruption, which leads ultimately to significant losses in food quality, including offflavour, off colours, poor texture etc. (Stanley, 1991).

One approach to enhancing oxidative stability of meat is to add antioxidants either into the animal diet or directly during processing (Decker, 1998). For example, an increasing body of evidence indicates that increased vitamin E supplementation is an effective means of meat quality improvement in chickens, turkeys, cattle, pigs and lambs (Sheehy et al., 1997; Wulf et al., 1995; Buckley et al., 1995; Liu et al., 1995). Therefore, vitamin E is proven to be effective in decreasing meat susceptibility during its processing and storage (for a review Surai and Fisinin, 2010). High dietary vitamin E supplementation (up to 250-500 mg/kg) is proven to be effective in improving meat stability. Therefore, the technology is developed and hundreds of publication proved the point, the only problem that is commercially not solved is who will pay for extra cost of feed due to high vitamin E concentration. In general, due to increased public awareness on negative effects of peroxide formed during meat processing, storage and cooking time will come that this technology will be in great demand.

Carotenoids could have a positive effect on meat quality during storage and there are some publications to substantiate this idea but due to limited accumulation of carotenoids in muscle, there is a need for further research in this field.

Selenium is proven to increase meat resistance to oxidation due to activation of various selenoproteins. However, this effect is related mainly to that area where feed ingredients are low in natural Se. Indeed Se-protein expression will not be increased due to additional Se supplementation beyond physiological needs (Surai, 2006).

Vitamin C alone is not effective as a feed additive to increase antioxidant defences in muscle, however, in various antioxidant combinations it could be effectively used.

Polyphenols are shown in various studies to affect meat quality but it seems likely that their direct addition to meat during processing is more effective. Indeed, our recent analysis of the role of polyphenols in poultry nutrition (Surai, 2013) indicated that the major direct effects of polyphenols is in the gut of the chicken and their concentrations in muscles are too low to have direct antioxidant effects. Therefore, the positive effects of various plant extracts fed to chickens, including improvement of meat stability, are, most likely, due to other mechanisms than their possible antioxidant action.

Resent research clearly showed that there is an opportunity to produce meat enriched with various nutrients, including Se and vitamin E (Surai, 2006; Fisinin et al., 2009). Indeed, in Ukraine there is a company (Landgut Ukraine) producing 20,000 tons of broiler meat enriched with Se and vitamin E, which is on sale in major supermarkets all over the Ukraine. In fact, 100 g such meat would deliver 50% RDA in selenium. Furthermore, increased vitamin E concentration in the meat substantially decreases meat susceptibility to oxidation during storage and cooking. This technology is patented and the company producing this kind of meat under the license bought in the British company Feed-Food.Ltd. Therefore, this technology combines the benefit of increased vitamin E concentration and gives an opportunity to solve Se-deficiency problems in many European countries, including the Ukraine.

Antioxidant Addition to Meat during Processing

Various natural and synthetic antioxidants are widely used during meat processing and a tendency is to replace synthetic antioxidants by natural compounds. Indeed vitamin E, carotenoids and polyphenols play an important role in improving meat stability during storage and cooking and in this area, polyphenol research is very promising. There were quite a few products tested as additives to processed meat. For example,

inclusion of 3% dried plum puree was effective as a natural antioxidant for suppressing lipid oxidation in precooked pork sausage patties (Nuñez de Gonzalez et al., 2008). Similarly, grape seed extract was shown to be an effective antioxidant in ground chicken thigh meat that does not affect moisture content or pH during storage, inhibits TBARS formation, helps to mitigate the prooxidative effects of NaCl, and may alter the effect of NaCl on protein solubility in salted chicken patties (Brannan, 2008). Indeed, grape seed extract at 0.02% has the potential to reduce oxidative rancidity and improve shelf life of refrigerated cooked beef and pork patties (Rojas and Brewer, 2007). The antioxidant and antimicrobial effects of equivalent concentrations of fresh garlic (FG), garlic powder (GP) and garlic oil (GO) were investigated against lipid oxidation and microbial growth in raw chicken sausage during storage at 3 degrees C (Sallam et al., 2004). Addition of either garlic or BHA (0.1 g/kg) significantly delayed lipid oxidation when compared with control. The results suggest that fresh garlic and garlic powder, through their combined antioxidant and antimicrobial effects, are potentially useful in preserving meat products. Furthermore, BHA/BHT, grape seed extract, pine bark extract and oleoresin rosemary added to the meat during its processing retarded the formation of TBARS by, 75%, 92%, 94% and 92%, respectively, after 9 days, and significantly lowered the hexanal content throughout the storage period (Ahn et al., 2007). Indeed, when plant extracts or individual flavonoids are added to the meat, their antioxidant properties can be easily observed as there is no problem with their absorption and metabolic changes.

A New Concept of Fighting Stresses

For the last few years, it has become clear that various stresses in poultry production are responsible for decrease of productive and reproductive performances of growing broilers as well as breeder performance. Recently it has been proven that oxidative stress is a molecular mechanism of many different stresses, including environmental stresses (e.g. heat stress), nutritional stresses (e.g. mycotoxins) and internal stresses (e.g. bacterial, viral, etc.) (Surai, 2002; 2006; Surai and Fisinin, 2012). Natural antioxidants (including vitamin E, carotenoids and selenium) are very important in maintaining antioxidant defences in chicken during embryonic and postnatal development. Recently a new concept of vita-genes regulating adaptive ability of human and animals to various stresses has been developed (Calabrese et al., 2008; 2010; 2012). In accordance with this concept in the body/ cell there is a range of genes responsible for synthesis various antioxidant compounds (heat shock proteins, antioxidant enzymes, sirtuins, etc.) and there are nutrients which can affect expression of such genes. Carnitine, betaine, vitamin E and some other elements are proven to be effective regulator of vita genes.

Based on aforementioned information about positive effects of dietary antioxidants on protection against various stresses in broiler production a range of antistress compositions/premixes has been developed and can be found on the market. However, the problem is that in stress conditions feed consumptions is substantially decreased and effect of any feed supplements decreased as well. The new concept was based on an idea that supplying birds with various antioxidants with water could help to deal with stress conditions more effectively. Indeed, it was proven that inclusion with water of vita-gene-regulating compounds (carnitine, betaine, vitamin E, etc.), as well as some minerals, vitamins, electrolytes and organic acids) could be effective in fighting various stresses (Surai and Fisinin, 2012a). This helps at stage of chick placement, when antioxidant system is crucial for the development of digestive and immune system (Fisinin and Surai, 2012a; 2011b). In particular, it was proven that inclusion of antistress composition (Antistress Magic Mix, Perfor-Max) into the drinking water at the University trial improved chicken growth and FCR (Fotina et al., 2011). Using the same antistress composition at commercial conditions improved FCR every week during 39-days broiler growth trial. At the end of the trial, the improvement in FCR due to usage of antistress composition during first three days post-hatch as well as before and after vaccination was highly significant (Velichko and Surai, 2013). In addition, it was shown that the antistress composition had an immunomodulating effects in broilers (Fotina et al., 2011) and growing ducklings (Fotina et al., 2012). Improvement of antioxidant system via supplying antioxidant composition via water could help dealing with various mycotoxins in feed, including DON (Fisinin and Surai, 2011c; 2011d), ochratoxin (Fisinin and Surai, 2012e; 2012f) and T-2 toxin (Fisinin and Surai, 2021g; 2012h). Furthermore, such technology could help with improving eggshell quality (Surai and Fotina, 2010) and preventing cannibalism (Surai, 2012c).

Conclusions

Taking into account information presented above it is clear that there is a great opportunity for broiler producer to use natural antioxidants in breeder diet to improve hatched chick quality as well as in broiler diet to maintain growth and immunocompetence. Furthermore, increased doses of various antioxidants in broiler diets could help to improve meat quality during processing, storage and cooking. Recently a new concept of vita-genes has been developed and there is

a proof that supplying various antioxidants via water medication could be an important and effective tool to manage stresses during broiler production. Clearly, more research is needed in this exciting area.

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