048 Invited Lecture

Importance of antioxidant balance for egg quality

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ANTIOXIDANT SYSTEM IN THE BODY

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. It has been calculated that about 2 x10^{10} molecules of reactive oxygen species (ROS) are generated per cell per day (for review see Surai, 2006) In stress conditions this rate is substantially increased.

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. These mechanisms are described by the general term "antioxidant system" (Surai, 2002). It is diverse and is responsible for the protection of cells from the actions of ROS. 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 and a thioredoxin system .

The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space, enabling maximum cellular protection to occur. The antioxidant system of the living cell includes three major levels of defence. The first level is based on the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase which, together with metal-binding proteins, are responsible for prevention of free radical formation and keep this process under control. The second level of antioxidant defence is based on chain-breaking antioxidants (vitamins E, C, carotenoids, etc.) and is responsible for restriction of chain formation and propagation. The third level of defence is based on the activity of specific enzymes, responsible for repairing or removal of damaged molecules from the cell.

The diet provides a range of natural compounds possessing antioxidant activity. For example, vitamin E comprises of 8 compounds: 4 tocopherols and 4 tocotrienols. The carotenoid family includes more than 600 compounds and there are more than 8,000 flavonoids. However, in the body, all antioxidants are working in concert as a team, the "antioxidant system", responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. In this team, every member has its own job to do and they are located in various parts of the cell in such a way to provide a maximal efficiency of antioxidant protection.

It is well known that vitamin E is the major antioxidant in biological systems, providing antioxidant defences at the membrane level. It can be found in practically all feed ingredients in varying concentrations. Vegetable oils are the richest source of vitamin E. However, because the oil refining process removes most of the vitamin E, commercially used oils (soya, maize, sunflower) are not high in this vitamin. The vitamin E requirement of poultry is in the range of 5-20 ppm. It is a general practise in poultry production to add vitamin E as a part of premixes. The level of supplementation varies substantially depending on country and conditions. For commercial laying hens vitamin E supplementation is in a range of 5-30 ppm. Vitamin E is not toxic for poultry. Recently, it has been shown that vitamin E can be effective only in an optimal balance with selenium (Se). Indeed, vitamin E is the main chain breaking antioxidant in the cell. Thus, vitamin E breaks the chain reaction of lipid peroxidation by removing lipid peroxyl radicals (LOO*). However, hydroperoxides (LOOH), produced in the reaction of vitamin E with the peroxyl radical, are toxic and if not removed, impair membrane structure and functions (Surai, 2002). In fact, lipid hydroperoxides are not stable and in the presence of transition metal ions can decompose producing new free radicals and cytotoxic aldehydes. Therefore, hydroperoxides have to be removed from the cell and only Se-dependent GSH-Px can deal with them, converting hydroperoxides into non-reactive products. Thus, vitamin E performs only half the job of preventing lipid peroxidation by scavenging free radicals and forming hydroperoxides. The second part of this important process of antioxidant defence is due to Se-GSH-Px. It is necessary to underline, that vitamin E and Se work in tandem; and even very high doses of dietary vitamin E cannot replace Se, which is needed (in the form of GSH-Px and thioredoxin reductase) to complete the second part of antioxidant defence as mentioned above. Thus, Se as an integral part of the GSH-Px and thioredoxin reductase, belongs to the first and second levels of antioxidant defence.

Se is considered to be an integral part of at least 25 selenoproteins expressed in various tissues of human and animals. It has been proven that Se participates in regulation of major physiological functions in human and animals including growth, development, spermatogenesis and embryonic development. Se can be found in major feed ingredients, but its concentration varies substantially and in many areas of the world, including Europe, Se concentration in grains is <0.1 ppm. The Se requirement of poultry is 0.1-0.2 ppm but common Se supplementation is in the range of 0.1-0.3 ppm. The legal limit in EU countries is 0.5 ppm of total feed derived+ supplemental) Se, while in the USA the supplemental Se level is restricted to 0.3 ppm. For the last few years it has been proven that a replacement of sodium selenite by organic Se in the form of Se-yeast is associated with improved chicken performance (Surai, 2006).

Recently, carotenoids have been included into the antioxidant family. In poultry production two major diets are used. They are the soya-maize based diet containing comparatively high levels of carotenoids and the wheat-barley-based diet that has a low carotenoid content. Lutein and zeaxanthin are major feed-derived carotenoids. There is no requirement for carotenoids established in poultry. However, there are some data indicating that carotenoids could have a role in chicken embryonic development. In wild birds, the egg yolk of many species contains 3-10-fold higher carotenoid concentration than in eggs of commercial chickens. Carotenoids have immunomodulating properties. Moreover, during embryonic development a portion of the carotenoids in egg yolk are used by the embryo, presumably in antioxidant reactions. However, whether birds have an absolute need for carotenoids remains to be established. In commercial table egg production carotenoids are used to achieve favourable egg yolk colour/

The roles of flavonoids and various flavonoid-containing compounds (herbs, plant extracts, etc) are not well understood and need further research. In particular, recently the idea of antioxidant-prooxidant balance in the digestive tract as a major determinant of human and animal health was put forward (Surai et al., 2003; 2004) and some important features of this balance in the chicken were investigated (McLean et al., 2005). Indeed, many antioxidant compounds such as flavonoids, which are not well absorbed can have profound health-promoting effects in the digestive tract preventing possible damage to enterocytes by various harmful compounds of the feed, including oxidised fat, mycotoxins and others. Recently, substantial attention was given to the microbial balance in the digestive tract and the possibilities for their regulation by various nutritional means. It seems likely that antioxidant-pro-oxidant balance is the second part of the same equation. Indeed, on the one hand, the bacterial population in the digestive tract depends on the diet used. On the other hand, bacteria can affect the antioxidant-prooxidant balance by triggering immune cell to produce free radicals.

NATURAL ANTIOXIDANTS AND EGG QUALITY

Major applications of natural antioxidants in table egg production include:

- improvement of egg production and quality in stress conditions
- improvement of egg quality during storage
- prevention of lipid and cholesterol oxidation during egg storage and cooking
- production of antioxidant-enriched eggs

It is well established that various stress conditions affect detrimentally egg production and internal egg quality. In this respect usage of vitamin E and selenium is of great importance. For-example, when flaxseed was fed to laying hens vitamin E (50 IU/kg) significantly improved egg production compared to 27 IU/kg (Scheideler and Froning, 1996). Percentage hen-day ovulation and percentage hen-day normal egg production during the late laying period (from 410 to 441 days of age) were significantly increased as a result of increased (300 IU/kg diet vs 10 IU/kg diet) vitamin E supplementation (Siegel et al., 2001). Moreover, performance characteristics and immune function of heat-stressed poultry have been shown to be improved significantly by increased fortification of vitamin E (El-Boushy, 1984). Supplementing diets of laying hens with a relatively high concentration of α-tocopheryl acetate (500 mg/kg) can reduce the detrimental effect of chronic temperature stress upon egg production (Bollengier-Lee et al., 1998). Even lower vitamin E doses (250 mg/kg diet) provided before, during and after heat stress were effective in alleviating the adverse effects of chronic heat stress in laying hens (Bollengier-Lee et al., 1999). Natural antioxidants can also help overcoming cold stress in birds. For example, 250 and 500 mg vitamin E/kg diet compared with 125 mg/kg diet and higher dietary selenium inclusions (0.2 vs 0.1 mg/kg) resulted in a better body weight, egg production, and feed efficiency in quails reared under cold stress. Similarly, egg weight, egg specific gravity, eggshell thickness, and Haugh units were positively influenced with vitamin E and selenium ($p <$ or $= 0.05$) supplementation (Sahin et al., 2003).

Vitamin E plays an important role in preventing lipid peroxidation in egg yolk during storage. Indeed reduced tocopherol content and higher thiobarbituric acid values have been shown in eggs stored over 10 days (Cherian *et al.*, 1996). Lipid manipulation of the diet may be responsible for the decreased vitamin E level in the egg yolk. Addition of up to 2.8% fish oil to the chicken diets increased the n-3 PUFA content of yolks with a concomitant imbalance between vitamin E and PUFA, leading to increased levels of cytotoxic aldehydic lipid peroxidation products (Grune et al., 2001). Therefore inclusion of fish oil in the hen's diet has been associated with a decreased vitamin E accumulation in the egg yolk (Cherian and Sim, 1997; Grune et al., 2001). In general lipid stability of egg yolk was significantly improved with increasing dietary tocopherol supplementation (Qi and Sim, 1998; Grune et al., 2001). Indeed, egg enrichment by vitamin E and carotenoids decreased cholesterol oxidation in egg lipids exposed to nitrogen oxide (Lai et al, 1996) or during egg powder preparation (Lai et al, 1996a). Vitamin E enrichment of the egg yolk prevents carotenoids from oxidation as well (Lai et al, 1996a).

It has been shown that organic selenium can also affect egg shell quality. For example, Paton and Cantor (2000a) showed increased shell breaking strength due to feeding organic Se to Babcock laying hens at 80 weeks of age. Furthermore, a partial or full replacement of sodium selenite by organic selenium in the form of Sel-Plex was shown to increase egg production, egg weight and weight of egg parts including shell, yolk and albumin (Rutz *et al.*, 2003). Organic selenium in the form of Sel-Plex increased yolk and white weights relative to control and trends toward improved egg production, weight and FCR were noted with organic selenium addition. Similarly, replacement of 50% selenite (total supplemental Se was 0.4 ppm) in the laying hen diet by Se-yeast was associated with a significant increase in egg shell weight and thickness (Klecker et al., 1997; 2001).

Selenium was found in all parts of the egg, including shell and membranes (Surai, 2006). In fact, the highest Se concentration was detected in the shell membrane and Se concentration in the shell was comparable to that in the albumin. Selenium concentration in quail shell ranged from 122.5 to 186.5 ng/g, which represented about 12% of total egg Se. Inclusion of Sel-Plex in the quail diet (0.5 ppm) significantly increased Se concentration in the shell (up to 231.2-458.3 ng/g). Therefore Se concentration in shells from control birds comprised 152.6 ng/g and was almost doubled in the Sel-Plex group, comprising 307.6 ng/g. Furthermore, in Se-enriched eggs the shell contained about 9.2% of the total egg selenium. This change could possibly affect shell structure. It has been recently appreciated that organic matrix of the egg shell is responsible for crystal formation and ultimately determines shell quality.

Egg freshness is one of the most important parameters determining consumer perception and demand. During storage, egg freshness decreases. This process is associated with biochemical changes in composition and structure of egg membranes. In experiments conducted in Japan (Surai, 2006), inclusion of Se-yeast in the layer diet at 0.3 ppm Se/kg increased GSH-Px activity in the egg yolk and white. The Haugh units were used as an indicator of egg freshness. The value was high on day 1 in both treatment and control groups with no difference due to Se supplementation. As time progressed, Haugh units of the control group declined sharply while the declination was more moderate in the treatment group. By day 7, it was evident that the Haugh unit is significantly higher in the treatment group. Similar data were reported by Rutz *et al.* (2003). In Brazil, Sel-Plex (0.1-0.3 ppm) was added on top of a commercial premix, containing 0.15 ppm Se from an inorganic source (Pan *et al.*, 2004). A significant improvement in Haugh units were observed as a result of organic selenium supplementation. (Rutz *et al.*, 2003). These results are in agreement with other data from the same authors (Pan and Rutz, 2003). The data have commercial significance indicating a possibility to improve egg quality maintenance during storage. Therefore, inclusion of organic Se in the diet of laying hens can be used to maintain quality during storage. Furthermore, inclusion of organic selenium in the chicken diet can also increase Se concentration in the perivitelline membrane. Indeed, inclusion of Sel-Plex in the commercial diet significantly increased the Se level in the perivitelline membrane (Surai, 2006). Therefore, this could be an additional mechanism of a positive effect of Sel-Plex on egg freshness during storage. The effect of Se on egg freshness probably depends on age of hens, composition of diet and conditions of egg storage.

By using appropriate sources of selenium and vitamin E it is possible to produce eggs, containing at least 50% RDA in Se (about 30 ug Se per egg) and daily requirement (15 mg) in vitamin E (Surai, 2002, 2006). These eggs could help to meet Se requirement in areas where Se deficiency is a real problem for general population. In fact whole Europe and many areas in Asia are considered to be Se deficient.

CONCLUSIONS

It seems likely that optimal combination of vitamin E and Se in the laying hen diet is an important determinant of egg production and quality.

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Structuring role of yolk and albumen in fresh egg pasta

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INTRODUCTION

Whole egg is widely used as a structuring ingredient in many food formulations. In fresh pasta, the presence of egg proteins gives a product with improved mechanical properties and cooking behavior (Alamprese et al., 2005b).

Commercial fresh egg pastas are formulated with a wide range of egg content (from 17 to 30%) and some products are manufactured with whole egg fortified with yolk. This high compositional variability affects mechanical and cooking properties of pasta (Alamprese et al., 2005a).

Aim of the present work was to study the influence of whole egg content and albumen to yolk ratio on structural characteristics of fresh egg pasta.

MATERIALS AND METHODS

Considering the egg amount (E) and the albumen to yolk ratio (A/Y) as factors, a Central Composite Design (CCD) was developed, varying E between 14.7 and 30.3% and A/Y between 0.18 and 5.82; the center sample was replicated five times. Fresh egg pasta was formulated with durum wheat semolina and soft wheat flour in a 1:1 ratio, adding enough water to maintain a constant dough moisture level (33%). Industrially pasteurized yolk and albumen products were used in different proportion in order to obtain the desired E and A/Y levels. A total of 13 fresh egg pasta samples was produced, in a fully randomized order to avoid systematic biases. Run order, sample identification and factor levels are reported in Table 1.

Fresh pasta samples were produced in sheets for lasagna, using an automatic plant for the production of retail-manufactured pasta and obtaining sheets of approximately 1 mm thick. All pasta samples were characterized as reported by Alamprese et al. (2005b), evaluating protein and lipid content, cooking behavior and mechanical properties both of raw and cooked pasta.

Table 1. Run order, sample identification and factor levels of Central Composite Design.

Run order	Sample	E (%)	A/Y
1	P30.3/3	30.25	3.00
2	P28/5	28.00	5.00
3	P22.5/3a	22.50	3.00
4	P22.5/0.18	22.50	0.18
5	P22.5/3b	22.50	3.00
6	P22.5/5.82	22.50	5.82
7	P22.5/3c	22.50	3.00
8	P17/1	17.00	1.00
9	P22.5/3d	22.50	3.00
10	P28/1	28.00	1.00
11	P22.5/3e	22.50	3.00
12	P14.7/3	14.74	3.00
13	P17/5	17.00	5.00

RESULTS AND DISCUSSION

The range of variation of sample protein content in dependence of E was small (14.3-16.4 g/100g d.b.). Since lipid was brought in by yolk only, fat content resulted highly affected by A/Y, varying between 1.1 (sample P17/5) and 9.0 g/100g d.b. (sample P22.5/0.18). Table 2 shows the results of cooking behavior and mechanical properties, evaluated by tensile tests carried out on raw and cooked pasta.