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FUNCTION OF ANTIOXIDANTS DURING AVIAN EMBRYOGENESIS

Abstract.

Development of avian embryo is running out of the mother's organism. That makes him sensitive to stress caused with incubation parameters disturbances. Consequence of disturbance of embryo's homeostasis is unphysiological increase of free radicals (FR) number. In life organisms the three levels antioxidative defense mechanism is functioning to protect lipids, proteins and nucleic acids against damages caused by FR. The first "preventive" level FR are neutralized with metalcoenzymes and enzymes cooperating with them like glutathione peroxidase, superoxide dismutase and catalase. The second "preventive – therapeutic" level is made by such substances like riboflavin (vitamin B2),L (+) ascorbic acid (vitamin C), tocopherol (vitamin E), retinol (vitamin A) moreover gluthatione and uric acid. This substances inhibit the initiation phase, remove reactive oxygen sorts (ROS) and also prevent spreading of damages (inhibition of propagation phase). The third level depends on repairing and reconstructing damaged molecules with for example heat shocks proteins (HSP).

Antioxidative mechanism's function during bird's embryogenesis was (has been) not fully checked yet. Based on previously acquired knowledge, it seems that it perform very important role. Evidence of this includes the fact that all the necessary antioxidant substances are a natural component of the egg (carotenoids, α-tocopherol, selenium) or their synthesis begins in the first days of embryonic development (eg, ascorbic acid, glutathione, superoxide dismutase). The deficit of these compounds leads to impaired development and/or death of the embryo. For example, huge deficiency of riboflavin causes rapid death of chicken embryos between 10 and 13 day incubation as a result of accumulation of fatty acids oxidation process's substrates, and hypoglycemia. Research on cognition of antioxidant system of avian embryos are carried out in numerous research centres, and the results obtained are applicable not only in animal science, but also in human medicine. Therefore, the aim of this work is to present the current state of knowledge on the role of antioxidant substances in bird embryo development.

Key words: *Avian embryo, antioxidants, vitamins, free-radicals*

Introduction.

Development of avian embryo outside the mother's body makes it susceptible to stress-inducing disturbances of the incubation. Following disturbances of embryo's homeostasis provides to unphysiological increase in the number of free radicals (FR). Reactive oxygen species (ROS) and FR are atoms, molecules or ions which have one or more unpaired electrons in the outer orbital and are characterized by very high

reactivity. They are present in live systems, mostly in the form of: superoxide anion (O_2•), hydroxyl radical (HO•), singlet oxygen and nitrozyl radical (NO•, nitric oxide II). Under physiological conditions, life organism continually generates O_2•, in response to the reduction of molecular oxygen. FR are often generated in the cells of life organisms to defend against viruses and bacteria. However, in the case of disturbance of homeostasis, FR can damage lipids, proteins and nucleic acids of the organism **[Fellenberg and Speisky 2006]**.

To prevent this organism evolved a three-level system of antioxidant defence. At the first "preventive" level FR are neutralized with metalcoenzymes and enzymes cooperating with them like glutathione peroxidase, superoxide dismutase and catalase. The second "preventive – therapeutic" level is made by such substances like riboflavin (vitamin B2), L (+) ascorbic acid (vitamin C), tocopherol (vitamin E), retinol (vitamin A) moreover glutathione and uric acid. This substances inhibit the initiation phase, remove reactive oxygen sorts (ROS) and also prevent spreading of damages (inhibition of propagation phase). The third level depends on repairing and reconstructing damaged molecules with for example heat shocks proteins (HSP). **[Buckiova et al 1998, Fellenberg and Speisky 2006].**

The content of various substances as vitamins in the egg is variable and depends mainly on the content of these ingredients in birds' feed. For example the level of vitamin in the chick egg (*Gallus gallus domesticus*) is the least sensitive to changes in dietary retinol (Vitamin A), whereas vitamin B2 in the egg corresponds to similar variations rapidly. Riboflavin in the egg reaches the highest level, when vitamin D, pantothenic acid (Vitamin B5), folic acid, biotin and vitamin B12 deficiency is in a large deficiency in the hens diet **[Bielańska-Osuchowska 1983]**. If the diet lacks vitamins, or for some reason they are not properly absorbed, there are deficient caused diseases for both adult birds and their offspring. Moreover radiation can reduce levels of antioxidants that are used for DNA repair because they are used for removing free radicals that arise owing to radiation as it happens in the Chernobyl region, where were detected dramatically reduced levels of some antioxidants in barn swallows (*Hirundo rustica*) and blue tits (*Parus caeruleus*) eggs **[Moller *et al* 2005, Mшller *et al* 2008].** Also persistent organic pollutants (POPs) existing in the marine ecosystems may cause chronic exposure effects in wild birds breeding what is linked with lover antioxidant rates in blood plasma and egg yolk as it happens in European shag (*Phalacrocorax aristotelis*) **[Murvoll *et al.* 2006].** The lack of any of necessary antioxidants in the egg can cause a significant reduction in hatching because of congenital defects, hatching problems, and increased death levels **[Naber 1993, Mazurkiewicz 2005, Peterka *et al.* 1997].**

Retinol (vitamin A)

Vitamin A (retinol) is a group of fat-soluble vitamins. Antioxidant occurs in 3 forms: alcohol (retinol), an acid (retinoic acid) as well as its esters. Vitamin A is only present in animal tissues (mainly in the liver where it is stored), in the plants it can be found only as a pro-vitamin β-carotene. Vitamin A assimilation by the animal organism is linked to the content of the respective amino acids in the diet, calcium and phosphorus and also shows antagonism in relation to vitamin C as ascorbic acid administration in the case of retinol deficiency significantly increases the risk of death

of chicks **[Borzemska 1984, Niemczak 1998, Mazurkiewicz, 2005]**. Also post-hatch exposure to environmentally relevant polybrominated diphenyl ethers (PBDEs') which are used to prevent fire and are found in several products, such as textiles, electronics, and building materials, of American kestrels (*Falco sparverius*) provides to lower plasma retinol, hepatic retinol and retinyl palmitate concentrations **[Fernie *et al.* 2005].** Vitamin A is responsible for proper vision, because it is component of the rhodopsin (light-sensitive pigment found in retinal rods). From it depends the proper development and functioning of epithelial tissues and bone, it is involved in the synthesis of carbohydrates, fats transformations and reduces susceptibility to infection also affects the normal appetite, growth, fertility and productivity of animals. Deficiency of this vitamin in the chicken egg occurs when the quantity of feed is insufficient layer has a chronic inflammation of the intestines, or worked factors affecting its distribution. At deep shortage of this substance chicks fall for 10^{th} day of hatching in due to disturbances in the development of the skeleton, the skull and spinal cord and then between 16 and 18 day due to disruptions in metabolism and disorders of the urinary system functioning **[Borzemska 1984, Niemczak 1998, Mazurkiewicz, 2005]**. Supplementation with retinyl palmitne in Japanese quail diet increase egg weight but neither egg production nor cholesterol levels are altered **[Ramalho et al. 2008].** Supplementation of retinoic acid and its methyl ester in chicks hatched from vitamin A shortage eggs determines normal growth in both groups, but the group supplemented with the first substance is blind, because the acid form of vitamin A does not take part in the formation of rhodopsin **[Tomphson 1969]**. The extreme shortage of vitamin A in the egg provides to death of almost all embryos around 48 days of incubation. At that time in normal embryos a well-developed vascular placenta can be seen, while in the eggs deprived of vitamin A placenta does not develop, or there is blood in the form of scattered fields. In this case, injection of vitamin A to eggs does not increase hatching levels, but prolongs the embryonic development for a few days **[Tomphson 1969, Peterka *et al.* 1997**]. Also alcohol can disturb embryonic cardiovascular development because of causing vitamin A deficiency in Japanese quail as it was showed by **Twal and Zile (1997)** Retinoic acid shows a large teratogenicity, given to the eggs at doses of 5 mg in physiological saline or 20 micrograms of oil is lethal for embryos regardless of day of incubation, while his lower dose given between 2 and 5 day development cause serious malformations and changes in the heart **[Tomphson 1969, Peterka *et al.* 1997]**. Toxicity of some forms of vitamin A is spreadly proved. The retinoic acid injection to the Japanese quail subgerminal space in early-stage affects specifically craniofacial morphogenesis. It cause abnormal development and growth of the central nervous system too**[Ru 1997]**.

Carotenoids

Carotenoids are antioxidant, biologically active pigments involved in several physiological process and provides a range of health benefits to variety of animals. Their importance for the development of avian embryos and nestlings is crucial. Animals can't synthesise carotenoids so they must acquired to them with food **[Karadas et al. 2005, Biard *et al.* 2005, Biard *et al.* 2006]**. Depression of dietary carotenoids deposited into barn swallow (*Hirundo rustica*) egg yolk can increase the

susceptibility of embryonic tissues to FR attack and with additive vitamin A and E deficiency can increase DNA damage caused by free radicals **[Ames 1983, Krinsky 1998, Moller *et al* 2005]**. Although parental ability to provide nestlings with a carotenoid-rich diet may enhance offspring fitness. Supplementation of egg-laying females of blue tit (*Parus caeruleus*) results in a significant increase in carotenoid concentration in egg yolk what provides that nestlings from eggs laid by this females have longer tarsi, faster development of the immune system, and grow bigger yellow feathers than unsuplemented ones **[Biard *et al.* 2005, Karadas *et al.* 2005]**. Also diet of new-hatched nestlings have an influence on birds development because carotenoids given to great tit (*Parus major*) and blue tit (*Parus caeruleus*) nestlings enhance yellow feather colour in great tit nestlings and increase in body mass for carotenoid – fed nestlings of both species. Opposite to birds after parents feed with supplemented diet there is no effect of carotenoids supply on immune function of nestlings fed with carotenoids. Similar situation can be observed in vitamin E levels. The first mentioned group don't change vitamin E concentration in blood whereas in carotenoid-feed blue tit nestlings plasma vitamin E concentration, increase **[Biard *et al.* 2005, Biard *et al.* 2006]**. Moreover carotenoids seems to have modulatory interactions with other members of the antioxidant system. Carotenoids supplementation in diet of hihi (*Notiomystis cincta*) not only increases their presence in egg yolk and nestling plasma but also lets to increase selenium concentration in egg yolk, increased retinol concentration and decreased α-tocopherol concentration in blue tit nestling plasma **[Ewen *et al.* 2006]**.

Tocopherol (vitamin E)

Under the name of vitamin E is hidden combination of many compounds from the group of tocopherols and the most important for birds is α-tocopherol. It is a potent antioxidant that protects other biologically important compounds and cellular structures from damage caused by FR. The presence of vitamin E increases the synthesis of ascorbic acid in tissues and with it raises the body's resistance to viral and bacterial infections. Tocopherols are also involved in the metabolism of protein - carbohydrate and response for the proper functioning of nerve cells and muscle. Vitamin E deficiency is usually not the result of too small quantity of this substance in the diet, but a reason of accumulation of oxidative compounds in the body of the bird. Also too high level of vitamin A in feed inhibits the absorption of vitamin E, what can lead to hipovitaminosis. A negative relationship is revealed between PBDEs (polybrominated diphenyl ethers) and liver tocopherol levels in shag (*Phalacrocorax aristotelis*) hatchlings too **[Murvoll *et al.* 2006]** moreover exposure of domestic duck (*Anas platyrhynchos*) eggs to PBDEs reduces levels of tocopherol in liver too what makes tocopherol to be a potential useful biomarker for exposure to PBDEs in bird species **[Murvoll *et al.* 2005]**. In poultry during embryonic development tocopherol deficiency results in high mortality between 3 and 5 day of incubation, as a result of increased permeability of blood vessels and yolk circulating disturbances. Chickens hatched from α-tocopherol deficiency eggs characterize with poor viability and a tendency to softening of the brain. Whereas symptoms of hypervitaminosis are not observed because even in quantities of 20,000 mg/kg vitamin E in based flock feed does not affect adversely the growth and health of poultry **[Mazurkiewicz 2005,**

Surai 2000]. In some birds species as the king penguin (*Aptenodytes patagonicus*) the yolk lipids are rich in n-3 fatty acids, which are potentially susceptible to peroxidative damage so the yolk contents and yolk to embryo transfer of antioxidants and lipid soluble vitamins is important. The concentration of vitamin E in the unicubated penguin egg is 155 ug/g yolk. Throughout the second half of the incubation period, vitamins E and A are took up from the yolk into the yolk sac membrane and later accumulated in the liver. Vitamin A is transferred in advance of vitamin E. After hatching, the penguin brain contains relatively little vitamin E (4.7 μg/g) compared to the much higher concentration in the liver (482.9 ug/g) at this stage. The yolk fatty acids of the king penguin can be example of potential oxidative susceptibility, because their yolk lipids differ in their degree of unsaturation **[Surai *et al.* 2001]**. Between days 16 and 22 of turkey embryo development, the α-tocopherol concentration in the liver remain constant and then increase reaching a maximum just after hatching. Similar changes are observed for the other tocopherols and tocotrienols. Although there is a discrimination between tocopherols and tocotrienols during their assimilation from the turkey diet by the parent hen and during metabolism by the developing turkey embryo **[Surai *et al.* 1999]**. Supplementation of vitamin E in the diet of hens livestock effects in increasing the content of this vitamin in the egg and appease the effects of oxidative stress by enhancing activities of antioxidant mechanisms during embryonic development and in the first days of chicks life. It also increases the resistance of embryos and affects hypothermia by raising antibodies in new – hatched offspring and influence positively on the results of breeding **[Niemczak 1998, Surai and Sparks 2001, Lin *et al.* 2005, Siegel *et al.* 2006, Viera 2007, Weber 2009]**. A supplementation of vitamin E in Japanis quail diet can provide better reproductive performance of male breeder whereas it does not affect fertility and hatchability of its male and female offspring **[Hooda *et al.* 2007]** whereas in blue tits breeding hatching success increase with increasing concentration of vitamin E **[Mшller 2008].**

Riboflavin (vitamin B2)

Riboflavin plays a key role in the metabolism of the organism as a source of flavin mononucleotide (FMN) and flawin adenine dinucleotide (FAD) **[Murray et al. 2008]**. At adoult birds deficience of this vitamin is very rare. The symptoms of such desease are lower egg yield and lighter egg white colour. Riboflavin deficiency usually occurs in chickens at the age of 2 - 4 weeks. In this case, growth retardation, progressive wasting and weakness with preserved appetite is observed. Toes become bent or make a fist. The skin is rough and cracking on fingers. Leg muscles disappear and the wings are lowered. In the corners of the beak and eyelids may develop inflammations **[Borzemska 1984, Kamienicki 2001, Mazurkiewicz 2005].** Especially form of vitamin B2 depression is the one caused by riboflavin binding protein gene mutation which encourage hens to deposit riboflavin in their eggs. Effects of riboflavin deficiency during embryogenesis are revealed only after the 10 day of incubation: the hypoglycemia and the process of accumulation of substrate oxidation of fatty acids begin. Embryo death occurs suddenly, about 13 day of incubation, and is preceded only by short-lived, lasting about 1 hour disturbance of the heart rate. The immediate cause of death appears to be the inhibition of many

flawin-dependent enzymes and an inability to carry out the fatty acids oxidation **[Borzemska 1984, Lee and White 1996]**. The effect of the rd gene can be abolished by in ovo injection of riboflavin. Supplemented embryos survive and develop normally, indicating that the unbound riboflavin in the egg protein can also be used during development. After hatching chicks take riboflavin from the feed and develop normally **[Lee and White 1996]**.

From the huge group of different antioxidants there is only a few substances depicted in this research. Although the exact effects of many antioxidant compounds including vitamins is still not fully understood, their positive effect on breeding, embryonic development and hatching of birds is huge **[Niemczak 1998, Lee and White 1996, Mazurkiewicz 2005]**. It also appears that these compounds may have beneficial effects in combating environment pollution. Antioxidants seems to lift the effects of compounds such as POPs like PBDEs **[Fernie *et al.* 2005, Murvoll *et al.* 2006]**, or even radioactive radiation **[Moller *et al* 2005, Mıller *et al* 2008]**. Therefore some researches should be taken to fully understand function and mechanisms of antioxidative substances activity.

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