α-TOCOPHEROL LEVELS IN DIFFERENT CHICK TISSUES IN RELATION WITH DIETARY INTAKE

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Key words: α-tocopherol, HPLC, dietary supplementation, chick tissue

Background

Food and food materials are deteriorated by oxidation of lipids during food processing or storage. The addition of antioxidants is one of the most effective ways to prevent the oxidation of the lipids in foods that contain fats and oils. Susceptibility of muscle food to lipid oxidation can be controled by the presence of antioxidants (Cheryl et al. 1996). The development of oxidative changes could be prevented by nitrites, phosphates and citrates, by natural and synthetic antioxidants, such as BHA, BHT and TVHQ. However, in the recent years, there has been increasing resistance to the use of synthetic antioxidants, since it has been established that some of them have a cancerogenic effect. That is the reason why today great attention is devoted to studies of antioxidative characteristics of natural compounds such as vitamin E, ascorbic acid, β-carotene, glutation, carnosine, anserine, rosemary extract or other plants (O'Neill et al. 1999). α-Tocopherol acts as a free radical scavenger, and its localization within the highly unsaturated bilayer of phospholipids of cell membranes provides controlle of lipid oxidation, improving at the same time quality characteristics of meat, such as color, flavor, texture, nutritive value, and other desirable sensory atributes, and consequently, extending its shelf life (Morrissey et al. 1994). The desired effects of the use of antioxidants *post mortem*, in the phase of technological preparation of meat is well known. However, difficult incorporation and dispersion limit their use. Contrary to this, the addition of antioxidants to cattle feed can increase their concentration in organs and tissue, and enable optimum distribution (Kočovski et al. 1998).

Objective

Lipids of poultry meat contain more unsaturated fatty acids than meat of large cattle, and therefore they are much more susceptible to oxidation. According to many findings there is no doubt that the addition of larger quantities of α-tocopherol in broiler feed results in a better effect on oxidative stability of meat. While the positive effect of vitamin E on the oxidative stability of lipids is doubtless it is still debatable what their optimum concentrations in cattle feed are. So, the aim of this experiments was to investigate the relations between dietary intake of α-tocopherol and its distribution in tissues of different chick tissues.

Methods

The broilers were devided into three grops. The first group, marked as control, was fed with common broiler feed. The second and the third group of broilers were fed with diets supplemented with α-tocopherol at 50 mg/kg and 75mg/kg of feed, respectively. The chickens were fed a single diet throughout the experiment which lasted 42 days.

Content of vitamin E expressed as α-tocopherol was determined in liver, thigh, and chicken breast according to Ayed et al. 1989; Leonhardt et al. 1997 and Sheehy et al. 1991. Samples were ground down and throughly mixed, avoiding heating of the sample. BHT was added as a protection against oxidation. The prepared samples were further processed immediately or stored in the frozen at –18 ^{0}C. Approximately 10 g of the prepared sample were weighed accurately to 0.01 g, transfered with 50 ml of the methanolic 0.5 % ascorbic acid solution (w/v) into a 250 ml round flask with reflux condenser. After addition of 10 ml of 50 % potassium hydroxide solution (w/v) the nitrogen was drawn through the condenser, and the mixture was heated to boiling on the water bath for 20 min. The still fairly warm content of the flask was filtered, and transfered into a 250 ml conical separating funnel containing 20 ml n-hexane. After phase separation the extraction was repeated twice with 20 ml n-hexane. The combined extracts were washed with water until free of alkalis. The extract was dried on anhidrous sodium-sulphate. The extract was then carefully evaporated to dryness in the rotary evaporator under a partial vacuum at a maximum of 40 ^{0}C water bath temperature. The dry residue was dissolved in 5 ml methanol (sample test solution) (Brubachter et al. 1986).

For the analyses of broiler feed (common and α-tocopherol supplemented) approximately 5g of sample was prepared in the same manner and finaly diluted in 10ml methanol (sample test solution).

Approximately 100 mg dl-α-tocopherol (Merck) was weighted to an accuracy of 0.01 mg, treated through the same procedure and made up to 100 ml with methanol to prepare stock solution with approximately 1 mg α-tocopherol/ml. The stock solution was further diluted in order to provide series of standards for calibration. The final concentrations of the calibration solutions were in the range of concentrations of α-tocopherol in test samples. Prior to injection all solutios were filtered through 0.45 μm Millipore filters. All reagents used were HPLC grade.

Chromatographic conditions:

Apparatus:	HP1090
Stationary phase:	Hypersil ODS, 100 x 2.1 mm i.d., 5μm
Injection volume:	10 μl (by loop)
Mobile phase:	Methanol
Flow rate:	0.150 ml/min
Temperature:	30 °C
Detection:	DAD, 294/4 nm, R 550/100 nm

The signal was evaluated by means of peak area (integrator). The calibration was checked repeatedly during the series of measurements every fifth injection with a double measurement using the standard test solution.

Results and discussion

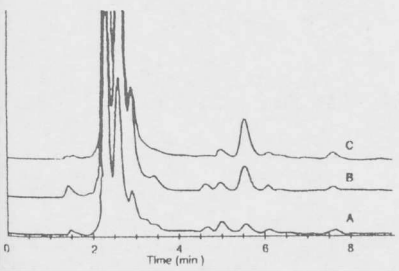

Fig.1. HPLC chromatograms of chick breast samples **A**) control; **B**) fed with 50 mg α-tocopherol/1 kg diet; **C**) fed with 75 mg α-tocopherol/1 kg diet.

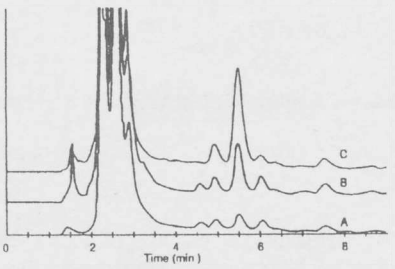

Fig.2. HPLC chromatograms of thigh muscle samples **A**) control; **B**) fed with 50 mg α-tocopherol/1 kg diet; **C**) fed with 75 mg α-tocopherol/1 kg diet.

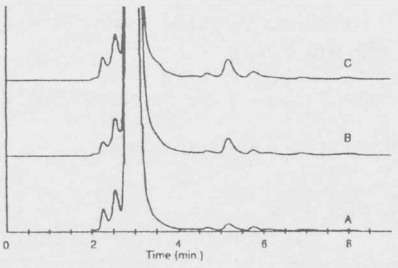

Fig.3. HPLC chromatograms of liver samples **A**) control; **B**) fed with 50 mg α-tocopherol/1 kg diet; **C**) fed with 75 mg α-tocopherol/1 kg diet.

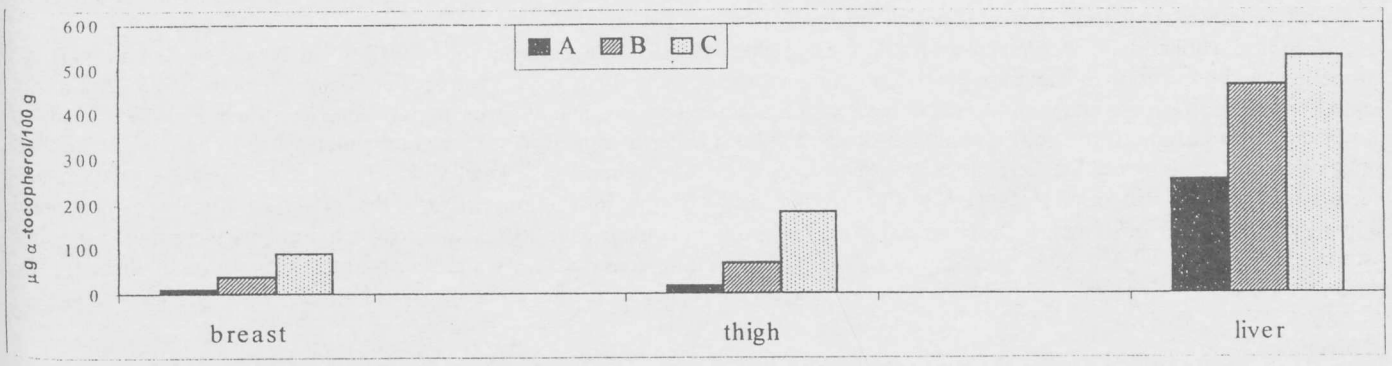

Fig.4. Content of α-tocopherol in different chick tissues, means **A** for control, **B**- fed with 50 mg α-tocopherol/1 kg diet; **C**- fed with 75 mg α-tocopherol/1 kg diet.

α-Tocopherol content in broiler feed without supplementation was 39.3 mg/kg diet. Supplemented quantities were confirmed. Results of chick tissues study (Fig.1-4.) show that: content of α-tocopherol in liver (1268 μg/100g) is greater than in thigh muscle (89.65 μg/100g), greater than in chicken breast (46.74 μg/100g) in chicks fed with feed without added α-tocopherol. Content of α-tocopherol increased 4.17 times in chicken breast (159 μg/100g), 3.87 times in thigh muscle (346.9 μg/100g) and 1.82 times in liver (2306 μg/100g) when the chicks were fed 42 days with feed supplemented 50 mg α-tocopherol/1 kg diet. Further increase in tissues was obtained with feed which contained 75 mg α-tocopherol/1 kg diet: 2.6 times was greater content of α-tocopherol in thigh muscle (903.5 μg/100g), 2.27 times in chick breast (443.2 μg/100g), 1.14 times in liver (2647 μg/100g) compared with results obtained by chicks fed with feed supplemented 50 mg α-tocopherol/1 kg diet.

Conclusions

This developed method was suitable for separation of α-tocopherol in broiler feed as well as in investigated chick tissues. Concentration of added α-tocopherol in feed cause concentration increase in tissues that improves its effective absorption in chosen range. The ability of α-tocopherol to influence risk for tissue injury and disease, mediated by its antioxidant activity, remains a hypothesis. Increase knowledge in this area of nutrition science will affect public health nutrition guidelines.

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