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## Poultry Products with Increased Content of CoQ<sub>10</sub> Prepared from Chickens Fed with Supplemental CoQ<sub>10</sub>

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### 1. Introduction

Nutritional science has become more and more focused on foods and food components that have the potential to optimize the physical and mental state of the consumer, as well as to reduce the risk of disease. In our laboratory, we aim to design food products for a healthy diet and to decrease the risk of suffering from chronic diseases, especially those that become more prevalent with advancing age.

Our emphasis is on food or food additives and feed with added coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>), or ubiquinone. CoQ<sub>10</sub> is a key component in the inner mitochondrial membrane, where it plays important role in oxidative phosphorylation (Lenaz et al., 2007). It is also present in other subcellular fractions and in plasma lipoproteins, where it acts as an important antioxidant (Bentinger et al., 2007). CoQ<sub>10</sub> has also been shown to have an effect on gene expression (Littarru & Tiano, 2007). These three functions are important for its use in clinical practice and in food supplementation.

CoQ<sub>10</sub> can be found in various foods in different concentrations. The richest nutritional sources of CoQ<sub>10</sub> are meat, fish, nuts, and some oils. CoQ<sub>10</sub> can also be found in vegetables, fruits, cereals, and dairy products but at much lower levels (Mattila & Kumpulainen, 2001). The average contribution from food sources is 3-5 mg of CoQ<sub>10</sub> a day for a healthy individual according to Weber et al. (1997). This amount can be easily consumed with normal food. In cases of deficiency, the contribution from food intake would have to be higher than 100 mg per day. This amount cannot be obtained only through food and therefore supplemental doses of CoQ<sub>10</sub> must be given to the patient. It is known that the absorption of exogenously administering CoQ<sub>10</sub> is slow and limited due to its lipophilic nature and relatively high molecular weight ( $M_r = 863$ ), which is consequently reflected in its relatively poor bioavailability. However, increasing its solubility in aqueous medium should, in most cases, increase its bioavailability, which is why, we developed a soluble

form of CoQ<sub>10</sub> that was later patented in the form of complex with  $\beta$ -cyclodextrin ( $\beta$ -CD) (Prošek et al., 2005). This new form of CoQ<sub>10</sub> exhibits increased water solubility and, consequently, better bioavailability in comparison to powder and oil-based CoQ<sub>10</sub> preparations (Prošek et al., 2008; Žmitek et al., 2008). This new form of CoQ<sub>10</sub> has already been commercialized as a functional food additive for human nutrition.

Our first selection for the practical use of this product was a group of dairy products (Stražičar et al., 2005). Their blend of fat and water was the ideal base for application of a functional food additive with an increased amount of CoQ<sub>10</sub>. Later, we found different meats (fish, poultry, beef and pork) and different kinds of pates suitable for fortification with water soluble CoQ<sub>10</sub>.

Our first attempt to evaluate the effect of CoQ<sub>10</sub> on living organisms was on free living non-laboratory broiler chickens. That study covered the accumulation of the CoQ<sub>10</sub> in different parts of the chicken's body (blood, heart, liver, and different muscle tissue) after different periods of administration (up to 40 days). For this purpose, 200 chickens, provenance ROSS 308, were used. Tests were carried out under optimal breeding and health conditions. Two different types of fodders (BRO-G and BRO-F2) were prepared in order to cover the needs of 40-day period. An adequate amount of water-soluble substance in the form of a paste with 7.5 % CoQ<sub>10</sub> was weighed and mixed in consecutive steps until a final concentration was obtained. The daily dose was set at 5 mg CoQ<sub>10</sub> per animal per day. For a better understanding of CoQ<sub>10</sub> accumulation, its distribution on the cellular level was studied by analyzing several subcellular fractions of breast muscle tissue (Jazbec et al., 2009).

We also tested the performance of poultry meat in industrial production with an increased quantity of CoQ<sub>10</sub> in an industrial scale experiment, where 37000 chickens were fed fodder enriched with CoQ<sub>10</sub> for the last 20 days before slaughtering. Fortified fodder was prepared as shown later in the text. In such a manner, we prepared natural functional meat products, which are more resistant to the potentially harmful effects of free radicals.

## 2. Ubiquinone

### 2.1 Properties of ubiquinone

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) is a lipid soluble molecule composed of a quinoid head and a hydrophobic tail, which contains 10 isoprenoid units (Fig. 1). It is an essential player in oxidative phosphorylation in the mitochondria and has an important role in the formation of ATP (Ernster & Dallner, 1995). It also maintains the fluidity of cellular and mitochondrial membranes and acts as an important antioxidant, which efficiently protects phospholipids, mitochondrial DNA, and membrane proteins from free radicals (Ernster & Dallner, 1995; Littarru, 1994; Crane, 2001; Bentinger et al., 2007)

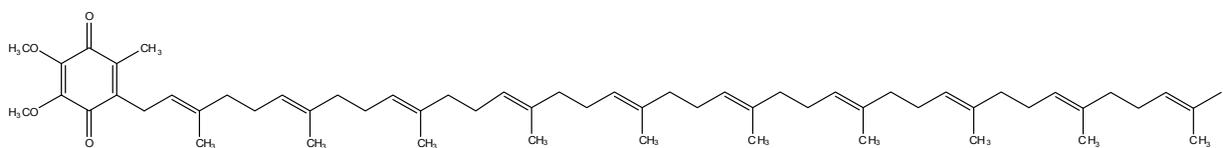


Fig. 1. Structure of CoQ<sub>10</sub> (in the form of ubiquinol)

## 2.2 Biosynthesis of ubiquinone

CoQ<sub>10</sub> is endogenously synthesized in all human and animal cells (Olson & Rudney, 1983; Elmberger et al., 1987). It can also be administered via dietary uptake through food and dietary supplements. The biosynthesis of CoQ<sub>10</sub> includes two pathways. The biosynthesis of the polyprenyl side chain runs through the mevalonate pathway. The reaction starts from acetyl-coenzyme A and ends up with farnesyl pyrophosphate (FPP). FPP is also the substrate for the biosynthesis of isoprenylated proteins, dolichol and cholesterol. The quinone head is synthesized from tyrosine and in some cases from phenylalanine (Turunen et al., 2004).

## 2.3 Intake of CoQ<sub>10</sub> with food and their absorption

CoQ<sub>10</sub> can be administered with plant and animal food. The richest sources of dietary CoQ<sub>10</sub> are meat and fishes, due to their relatively high levels of mitochondria. Dairy products and vegetables are much poorer in CoQ<sub>10</sub>, when compared to animal tissues. The average daily intake of CoQ<sub>10</sub> is estimated to be under 10 mg (Weber et al., 1997; Mattila & Kumpulainen, 2001).

Due to the lipophilic nature of CoQ<sub>10</sub> its absorption follows the same process as that of fat-soluble nutrients in the gastrointestinal tract (Bhagavan & Chopra, 2006). Following absorption, CoQ<sub>10</sub> is first incorporated into chylomicrons and is taken up rapidly by the liver where CoQ<sub>10</sub> is repackaged into very low density protein/low density protein particles (VLDL/LDL) and released into circulation (Kaikkonen et al., 2002).

## 3. Complex of CoQ<sub>10</sub> with $\beta$ -cyclodextrine

CoQ<sub>10</sub> is classified as a lipophilic compound and is practically insoluble in aqueous solutions. Due to its high molecular weight and poor water solubility, it is absorbed from the gastrointestinal tract poorly and slowly. Mainly soft and hard gel capsules filled with powder or CoQ<sub>10</sub> dispersed in sesame or soybean oil are available as a nutritional supplements on the market. Therefore, it has been a challenge to develop a CoQ<sub>10</sub> formulation for oral administration with better water-solubility and therefore better bioavailability.

Different methods have been used to improve the solubility of CoQ<sub>10</sub>. Some approaches have included the preparation of nanoparticles incorporating CoQ<sub>10</sub> (Hsu et al., 2003; Ankola et al., 2007), solubilization in a blend of sorbitan monooleate, polysorbate 80, medium chain triglycerides, propylene glycol,  $\alpha$ -tocopherol and poly vinyl pyrrolidone (Chopra et al., 1998), preparation of redispersible dry emulsion (Takeuchi et al., 1992), solid dispersion of CoQ<sub>10</sub> with Eudragit® (Nazzal et al., 2002), and fine oil-in-water emulsion via the development of a self-emulsifying drug delivery system (Kommuru et al., 2001). The most recent preparation of CoQ<sub>10</sub> is All-Q® 10% CWS/S, which is based on food grade starch (Ullmann et al., 2005). Some of the most useful enzyme-modified starch derivatives are cyclodextrins, which are well known as inclusion-complexing agents for small and large molecules (Szejtli, 1998).

The most common cyclodextrin (CD) is  $\beta$ -CD, a ring molecule consisting of seven glucopyranose units. Having a hydrophilic outer surface and a hydrophobic inner cavity

gives it a unique ability to form inclusion complexes with lipophilic compounds and increase their water-solubility, stability and/or bioavailability (Doorne, 1993). Lutka and Pawlaczyk (1995) tried to synthesize inclusion complexes of CoQ<sub>10</sub> with various CDs. They prepared inclusion complexes of CoQ<sub>10</sub> with  $\gamma$ -cyclodextrin ( $\gamma$ -CD) and substituted  $\gamma$ -CDs using “kneading” and “heating” methods, but they could not confirm a complex with either non-substituted  $\beta$ -CD or with  $\beta$ -CD.

In the Laboratory for Food Chemistry at the National Institute of Chemistry Slovenia, we participated in the development of a water-soluble form of CoQ<sub>10</sub>, which could be used in the preparation of functional foods. For this purpose, we prepared complexes of CoQ<sub>10</sub> with  $\beta$ -CD by a co-precipitation method in aqueous solution (Fig. 2). The complex of CoQ<sub>10</sub> with  $\beta$ -CD was patented (Prošek et al., 2005). The physicochemical characteristics of the resulting complexes, such as solubility in relation to temperature and pH, and the influence of temperature and ultra-violet (UV) light on its stability were also examined (Fir Milivojević et al., 2009 a, 2009 b).

The prepared complex of CoQ<sub>10</sub> with  $\beta$ -CD was characterized and quantified using chromatographic and spectroscopic techniques; thin layer chromatography (TLC), high performance liquid chromatography (HPLC), high performance liquid chromatography coupled with mass spectrometry (HPLC/MS), infra red spectrometry (IR), nuclear magnetic resonance (NMR). Among these sophisticated techniques, the relatively simple and inexpensive TLC gave us some very important and informative results. Identification and quantification of CoQ<sub>10</sub>/ $\beta$ -CD were done with three different TLC procedures (Prošek et al., 2002), one and two dimensional TLC (Prošek et al., 2004).

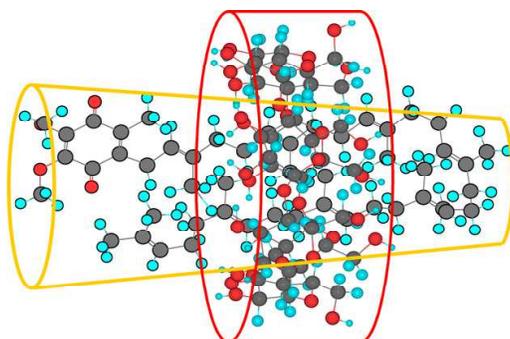


Fig. 2. Expected 3D structure of the inclusion complex of  $\beta$ -CD and CoQ<sub>10</sub> with folded isoprene chain

To prove the enhanced bioavailability of the CoQ<sub>10</sub>- $\beta$ CD complex, we performed two bioequivalence studies. In the first study, the relative bioavailability was investigated for two forms: our water-soluble CoQ<sub>10</sub> formulation and a commercially available oil-based CoQ<sub>10</sub> in the form of soft-gel capsules. The bioavailability was determined by measuring the plasma CoQ<sub>10</sub> levels periodically after administration to a group of beagle dogs. The mean value for the baseline plasma concentrations, maximum plasma concentrations ( $C_{max}$ ), time of maximum plasma concentration ( $T_{max}$ ), area under the plasma concentration curve  $AUC_{(0-48h)}$ , and the elimination half life ( $t_{1/2}$ ), were determined for both formulations. The results of the experiment show the advantage of water-soluble CoQ<sub>10</sub> over commercially available soft-gel capsules (Fig. 3). This is shown by the nearly three times higher  $AUC_{(0-48h)}$ , nearly two times higher  $C_{max}$ , and the shortened  $T_{max}$  from 6 to 4h, where  $AUC_{(0-48h)}$  represents the

area under the plasma concentration curve,  $C_{\max}$  the maximum plasma concentration, and  $T_{\max}$  the elimination half life (Prošek et al., 2008).

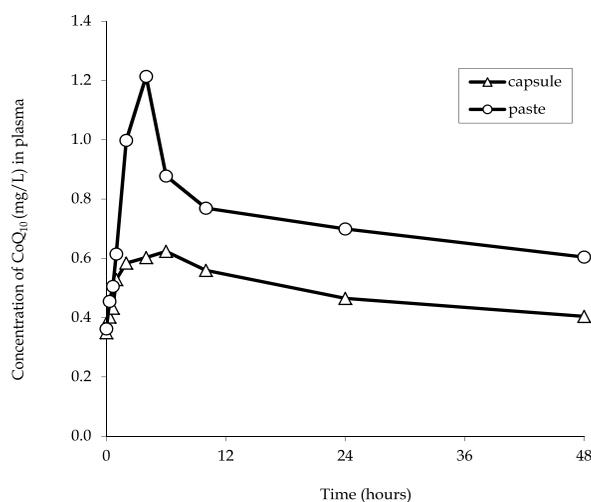


Fig. 3. Comparison of CoQ<sub>10</sub> absorption in oil capsules and in complexed CoQ<sub>10</sub>- $\beta$ CD in plasma (Prošek et al., 2008)

A second bioequivalence study was also performed for two formulations, a novel CoQ<sub>10</sub> paste with increased water-solubility and to soft-gel capsules with CoQ<sub>10</sub> in soybean oil, but this time on human subjects. This single-dose bioequivalence study once again revealed, that the oral absorption and bioavailability of CoQ<sub>10</sub> can be significantly affected by increasing the water solubility with the formation of CoQ<sub>10</sub>- $\beta$ CD complex, because it demonstrates superior bioavailability over the soft-gel capsules (Žmitek et al., 2008).

### 3.1 Food with added complex CoQ<sub>10</sub>- $\beta$ CD

The experiments showed that complex CoQ<sub>10</sub>- $\beta$ CD can be easily and uniformly mixed with many food products. We found that the most appropriate foods for this purpose are dairy and meat products. These kinds of products can be easily used to increase the daily intake of CoQ<sub>10</sub>, especially for persons having problems with digesting fats and vegetable oils, which are normally used as a matrix in non water soluble preparation of CoQ<sub>10</sub>. Additionally, when inclusion complex CoQ<sub>10</sub>- $\beta$ CD is placed into an environment with a pH below 3, it is disintegrated and CoQ<sub>10</sub> is released from the  $\beta$ -CD carrier in its natural form. This is the reason why a water-soluble substance in the form of a paste with 7-10% of CoQ<sub>10</sub> is used as a very efficient dietary supplement. It is also suitable for animals and humans who dislike or cannot swallow relatively big capsules.

#### 3.1.1 Dairy products fortified with CoQ<sub>10</sub>

To establish which kind of food products are most suitable carriers for our new additive we investigated different kinds of dairy products. Nearly 100 different products from retail stores were selected and analyzed, as we needed to know the approximate amount of CoQ<sub>10</sub> in different milk products. CoQ<sub>10</sub> was extracted with a combination of ethanol - n-hexane

extraction and analyzed using HPLC-MS. In Table 1 shows the concentration of CoQ<sub>10</sub> in milk produced in different Slovenian regions, with different amounts of fat and production procedures. The results were measured in the oxidized state as ubiquinone and represent the total amount of CoQ<sub>10</sub> in the samples. Unfortunately, few studies with reliable results have been published with which we could compare our results. The analyzed dairy products were divided into 6 groups: milk, yogurts, sour milk products, curds, creams and soybean "milk" products. From the obtained measurements it is evident that milk with higher amounts of fat has a higher amount of CoQ<sub>10</sub> and that sterilized milk prepared from concentrates has a lower amount of CoQ<sub>10</sub>, regardless of the amount of fat in the milk.

<b>Food products</b>	<b>Fat (%) declared</b>	<b>CoQ<sub>10</sub> (mg/kg)</b>	
<b>Milk</b>	Fresh cow milk from local farm	3.6	1.90
	Cow milk from the alpine region	3.5	1.57
	Cow milk from the alpine region	1.6	0.66
	Ultra-heat-treated homogenized milk	3.5	1.70
	Ultra-heat-treated homogenized milk	1.6	1.16
	Ultra-heat-treated homogenized milk	0.5	0.46
<b>Yogurt product</b>	Yogurt from goat and sheep milk	6.0	0.32
	Yogurt	3.2	1.13
	Yogurt	1.3	0.70
	Yogurt with fruits	3.2	0.72
	Yogurt	2.8	0.78
	Liquid yogurt	1.6	0.76
	Yogurt bioactive	1.5	1.36
	Yogurt bioactive with inulin	1.5	0.86
	Light yogurt with Ca	0.1	0.24
	Yogurt without fat	0.0	0.06
<b>Sour milk products and probiotics</b>	Sour milk	0.1	0.0
	Sour milk	3.2	0.51
	Sour milk	1.6	0.50
	Liquid sour milk	3.6	0.55
	Acidofil drink	3.2	0.91
	Probiotic drink	2.0	0.60
	Kefir	3.5	0.94
Kefir	1.6	0.68	
<b>Curd and cream products</b>	Cream	35.0	0.92
	Sour cream	21.5	0.90
	Cream for coffee	10.0	0.11
	Curd	34.0	0.68
	Curd pressed	13.4	0.68
<b>Soy milk and soybean product</b>	Soy drink	-	0.03
	Soy yogurt	-	0.04
	Soy yogurt with fruit	-	0.03

Table 1. Concentrations of CoQ<sub>10</sub> in some milk and milk products, as well as soy milk products

From acquired results is evident that milk with higher concentration of fat has also higher amount of CoQ<sub>10</sub> and that sterilized milk prepared from concentrates has a lower amount of CoQ<sub>10</sub>, regardless of the fat concentration.

The second group shows the concentrations of CoQ<sub>10</sub> in different kinds of yogurts. The concentrations of CoQ<sub>10</sub> vary from 1 mg/kg in yogurt from natural cow's milk with 3.2% of fat to practical zero in special light versions with no declared fat (0%). The sample preparation and extractions were not very effective, due to different types of additives in some yogurts, especially of plant origin, such as inulin and other sugar polymers. Our results show that yogurts produced from natural cow's milk with standardized amounts of fat (3.2%) are the right origin for dietary CoQ<sub>10</sub>. In the sour milk group, the kefir and acidophilous drinks' amount of CoQ<sub>10</sub> vary from sample to sample, but it is evident that the amount of fat correlates with the amounts of CoQ<sub>10</sub>. The next group shows CoQ<sub>10</sub> concentrations in creams and curds. The measured concentrations are not very high, especially if compared with the concentrations of fat in the samples. These types of milk products are not very suitable sources of natural CoQ<sub>10</sub>, as the consumer would have to eat too much fats. On the other hand, this type of food, mostly used as a desert, is very good for enrichment with herbs and CoQ<sub>10</sub>. Several soybean products were also analyzed, but we found only small amounts of CoQ<sub>10</sub> in purchased soy bean drinks and yogurts.

The measured values together with consumers' nutritional habits show that the milk-based dairy products are very convenient candidates for supplementation. Milk products with a suitable combination of fat and water are an ideal base for functional food products containing increased amounts of CoQ<sub>10</sub>. Ubiquinone is a fat soluble substance and a certain amount of fat in the carrier food is advantageous.

### 3.1.2 Pates fortified with CoQ<sub>10</sub>

The second set of food products which can easily be enriched with water soluble CoQ<sub>10</sub> are different meats (fish, poultry, beef and pork) and liver pates. A certain group of such products were purchased from local stores and analyzed. In addition to the concentrations of CoQ<sub>10</sub>, the total amount of fat, fatty acid profiles, and cholesterol were also determined.

The analyzed samples may be sorted in five groups. Quantitative results are shown in Table 2. In the first group, typical pork pates are shown; these usually contains: 10-25% pork liver, 5-30% pork meat, up to 10% pork fat, as well as ham, bacon, intestines, proteins, and carbohydrates. Results show a very high amount of CoQ<sub>10</sub>. The mean value of 9 different samples is 12.3 mg/kg, and the maximum value 27.4 mg/kg of CoQ<sub>10</sub>. There is a high level of extracted fat, with a mean value of nearly 24%. In the extracted fat, 40.3% is saturated fatty acids and only 17.7% is unsaturated, but nearly 40% is C18:1, which makes these products quite resistant to fat oxidation. On the downside, these types of pates do have high amount of cholesterol, with an average value of 0.46 g/kg.

The second group contains pates prepared from poultry meat and liver, with additives in the form of milk and soy proteins, vegetable fats, and hydrolyzed carbohydrates. This group showed the highest amount of CoQ<sub>10</sub> from among the entire group of analyzed samples. The mean value of CoQ<sub>10</sub> in the 9 different pates is 17.9 mg/kg. The level of total fat is not high but is very variable: the mean value is 19.9% and the maximal value is 30.8%. The variability is probably the result of different concentrations of vegetable fat added to the final products.

The amount of saturated fatty acids in fat is only 26.4%. The combination of C18:1 and other unsaturated fatty acids means there are up to 72.4% unsaturated fatty acids in these poultry products. But once again, as in the first group, the main drawback is the very high level of cholesterol, with an average value of 0.73 g/kg. This high value is probably the result of the high amount of fatty poultry skin in the product.

Pate	Pork pate	Poultry pate	Pork pate with vegetables fats	Beef pate	Fish pate
Components	Pork liver, fat, meat	Poultry meat, liver	Pork meat, vegetable fats & additives	Beef meat	Fish meat
<b>Saturated fatty acids (%)</b>	40.3	26.4	39.6	33.1	22.5
<b>Unsaturated fatty acids (%)</b>	17.7	39.7	16.0	27.6	53.0
<b>C18:1 (%)</b>	39.7	32.7	42.2	36.9	24.2
<b>Unsaturated fatty acids (sum total) (%)</b>	57.5	72.4	58.2	64.5	77.2
<b>Fat g/100 g</b>	23.9	19.9	25.5	21.2	17.0
<b>CoQ<sub>10</sub> g/100 g</b>	1.23	1.79	0.64	0.98	0.63
<b>Cholesterol g/100 g</b>	0.046	0.073	0.031	0.072	0.050

Table 2. Quantitative results of fat, fatty acids, CoQ<sub>10</sub>, and cholesterol in some meat and liver pates

In the third group, pates produced from pork meat and added vegetable fat and carbohydrates are presented. In this group the total amount of fat is high and its concentration is stable. The main reason is the controlled addition of vegetable fat, which also influences the amount of C18:1 and not the high amount of cholesterol, where the average value is only 0.3 g/kg. These positive effects are reduced by the relatively low amount of CoQ<sub>10</sub>, averaging only 6.4 mg/kg.

The fourth group consists of samples prepared from beef meat. These pates have small amount of fats, only 21.2%. The level of unsaturated fatty acids is relatively high, the level of CoQ<sub>10</sub> is acceptable, with an average value of 9.8 mg/kg, but the concentration of cholesterol is very high, with an average value of 0.7 g/kg.

In the last group are pates prepared from fish meat. Although only a small number of products were analyzed we can conclude from the obtained results that this group has a low amount of CoQ<sub>10</sub>: the mean value of 4 different pates is 6.3 mg/kg and the maximum value is 8.4 mg/kg. The level of total fat is very low, only 17.0%. The amount of saturated fatty acids is small (22.5%) and the amount of unsaturated fatty acids is extremely high (53.0%), if we include C18:1 it can reach up to 77.2%. The level of cholesterol is low, with an average value of 0.50 g/kg.

Results show that meat and liver pates can be a very good dietary source of CoQ<sub>10</sub>. The concentration is higher than in any other food products, such as in raw milk where the concentration of CoQ<sub>10</sub> is less than 3 mg/L. The only problem is the high amount of cholesterol, which is correlated with amount of CoQ<sub>10</sub>. In a product with a high amount of

ubiquinone there is also a high amount of cholesterol present. The relation between CoQ<sub>10</sub> and cholesterol in analyzed samples is shown in Fig. 4.

A strong correlation between CoQ<sub>10</sub> and cholesterol was found; however, there was no correlation between total fat and CoQ<sub>10</sub> and total fat and cholesterol. This is logical due to the different product formulation of meat pates even in cases where they have similar labels.

The obtained results show that CoQ<sub>10</sub> in meat pates is a reasonable supplement. It can change the ratio of CoQ<sub>10</sub> to cholesterol present in the meat products and makes them more suitable for the consumer.

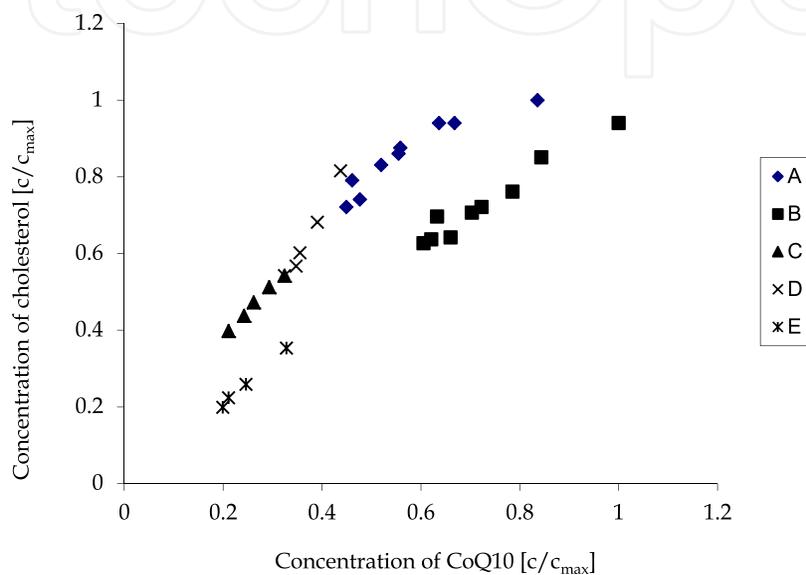


Fig. 4. Relation between concentrations of cholesterol and concentrations of CoQ<sub>10</sub> in different pates, expressed as the ratio between measured value and the max value on each coordinate ( $c_{\max}(\text{CoQ}_{10}) = 2.56 \text{ mg}/100 \text{ g}$ ,  $c_{\max}(\text{cholesterol}) = 201 \text{ mg}/100 \text{ g}$ ). Group A consists of pork liver, fat and meat; B of poultry meat and liver; C of pork meat, vegetable fats and additives; D of beef meat; E of fish meat

#### 4. Poultry products with increased content of CoQ<sub>10</sub>

The distribution of exogenous CoQ<sub>10</sub> in different tissues has been described mostly in rats and mice (Kwong et al., 2002; Kamzalov et al., 2003). In both rats and mice, the predominant endogenous form of CoQ is CoQ<sub>9</sub>, due to their relatively short life span. In humans and animals with longer life spans, including chicken, the major homologue is CoQ<sub>10</sub>. There is a lack of studies on feeding long-lived animals with dietary CoQ<sub>10</sub> and its distribution in tissue, which may explain the accumulation of CoQ<sub>10</sub> in animals whose predominant form of CoQ<sub>10</sub>. Until now, there was also not a lot of information available to describe the effect of long term controlled supplementation with CoQ<sub>10</sub>.

##### 4.1 Influence of added CoQ<sub>10</sub> in chicken feed on breeding and its accumulation in chicken tissue

In this study, we present the influence of CoQ<sub>10</sub> used as a food additive on the health and physical condition of chickens during the feeding period. The amounts of CoQ<sub>10</sub> and

cholesterol in chickens' blood before slaughtering and in different tissues after slaughtering are determined.

#### 4.1.1 Animals and experimental design

Two hundred 1-day-old ROSS 308 male chicken broilers were provided from a local hatchery (Perutnina Ptuj d.d., Slovenia). The study was carried out under optimal health and growing conditions, according to the prepared protocol. During the 40-day production period all animals were treated under identical controlled environmental and growing conditions with deep litter technology, except for the feed. One hundred chickens were treated as the control group (G0) and the other hundred (the group in the study) were distributed into four subgroups (G1-G4). The control group of animals was fed plain feed for the whole period, while the study group received feed fortified with water-soluble CoQ<sub>10</sub> paste with 7.5% of CoQ<sub>10</sub> in the form of inclusion complex with  $\beta$ -cyclodextrin. The active substance was synthesized in our laboratory (Laboratory for Food Chemistry, National Institute of Chemistry, Ljubljana, Slovenia) and mixed with the fodder in two steps in order to give a final concentration of 0.0042% CoQ<sub>10</sub>. The concentration of CoQ<sub>10</sub> in feed was calculated so that each animal received an average of approximately 5 mg of CoQ<sub>10</sub> per day. The subgroup G4 was fed with fortified feed for the whole period of 40 days. The subgroups G1, G2, and G3 were fed with fortified feed for the last 10, 20 and 30 days of the 40-day production period, respectively (Table 3).

Group of chicken	G0	G1	G2	G3	G4
Start of feeding (on day of age)	/	30 <sup>th</sup>	20 <sup>th</sup>	10 <sup>th</sup>	0 <sup>th</sup>
Time of feeding with CoQ <sub>10</sub> (number of days)	/	10	20	30	40
Content of CoQ <sub>10</sub> in feed (mg/day)	/	5	5	5	5
Number of chicken	109	25	25	25	25

Table 3. Experimental design of feeding chicken with added CoQ<sub>10</sub>

At the end of day 40, before slaughtering, blood samples from 20 randomly chosen birds were collected in commercially available heparinized tubes, centrifuged at 3000 g for 15 min and immediately frozen at -80 °C. After slaughtering, 6 birds were randomly chosen from each of the subgroups and several chicken parts (heart, liver, breast, leg, wing and body fat) were separately sampled, labelled, packed, and frozen at -20°C. After about 24 hours the collected frozen samples were transported to the laboratory facility for long-term storage at -80°C and kept so until needed for analysis.

#### 4.1.2 Growth of chicken

During the 40 day growth period, chickens were monitored by measuring their body weight and by feed intake weighing. Significant changes were noticed in the physical condition of chickens over the growth period of 40 days. In Fig. 5, the increase in the chicken weight is shown. The net increase in chickens weight in each group is compared to the average value of the control group at the corresponding measurement time. In this way the influences of higher starting weight of the control groups and non-uniform distribution of groups formation (10, 20, 30, 40 days) and weighting times (10, 21, 29, 36, and 40 days), are eliminated. Our study showed that a major increase in chicken body mass was seen in group

G4, followed by group G2. From the obtained results, we can conclude that the most economical results would be obtained with non-stop twenty days foddering.

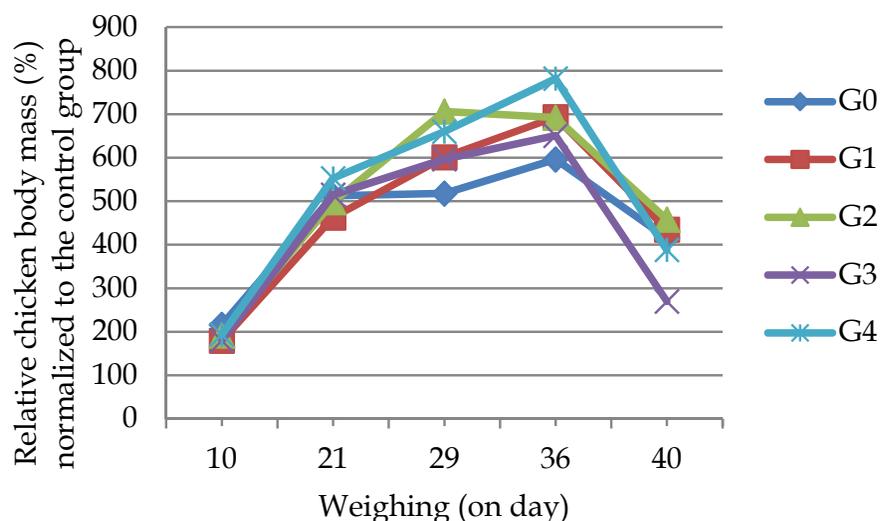


Fig. 5. Influence of exogenous CoQ<sub>10</sub> on chicken body mass (g) in groups (G0, G1, G2, G3 in G4) on day 10 (1<sup>st</sup> weighing), on day 21 (2<sup>nd</sup> weighing), on day 29 (3<sup>rd</sup> weighing), on day 36 (4<sup>th</sup> weighing) and on day 40 (5<sup>th</sup> weighing) of chicken age

We also observed that the addition of CoQ<sub>10</sub> had a strong influence health during breeding. Due to their fast growth, chickens are vulnerable to a number of diseases. Ascites is the most common metabolic disease in chicken and occur worldwide, especially at high altitudes. The disease has a complex aetiology and is predisposed to by reduced ventilation, and respiratory disease (Currie, 1999). The literature data has demonstrated the positive effect of exogenous CoQ<sub>10</sub> in reducing ascites mortality in broilers (Geng et al., 2004).

#### 4.1.3 Accumulation of CoQ<sub>10</sub> in plasma and various tissues

Four different groups of broiler chicken were administered CoQ<sub>10</sub> for the last 10, 20, 30, and 40 days prior to slaughter, after which the amounts of CoQ<sub>10</sub> and cholesterol were measured in plasma, liver, heart, breast, wings, and legs. The results for CoQ<sub>10</sub> (Fig. 6) and cholesterol concentrations (Fig. 7) in different samples from chickens were obtained by HPLC-MS and TLC methods, respectively.

After ingestion of feed enriched with CoQ<sub>10</sub>, molecules of CoQ<sub>10</sub> were transferred with other lipids via chylomicrons to liver cells. The results showed that the concentration of CoQ<sub>10</sub> in the liver after feeding with feed fortified with CoQ<sub>10</sub> in the various test groups is not statistically significantly changed in comparison to the control group. The liver, probably through unknown mechanisms, regulated the concentration of endogenously synthesized CoQ<sub>10</sub>. In liver tissue cholesterol concentration increased.

In the liver, CoQ<sub>10</sub> was incorporated into lipoproteins, mostly into VLDL/LDL particles and released into the circulation. The major function of CoQ<sub>10</sub> in the blood is as an antioxidant. CoQ<sub>10</sub> protects LDL from lipid peroxidation by scavenging peroxy radicals (Alleva et al., 1995, 1997). The antioxidant protection is important for broiler chickens

because of their rapid growth and consequent higher feed intake per time-unit and higher metabolic rate.

The concentration of CoQ<sub>10</sub> in plasma increased in all test groups by approximately 1.6-fold over that in the control group. CoQ<sub>10</sub> levels in blood also exhibited a noticeable maximum increase of about 80% in the case of group G3. However, the concentrations declined with prolonged, 40-day supplementation (group G4) to 140% of the levels found in the control group (G0). In the case of blood levels, a variety of values within each group were found, leading to large differences in the relative standard deviations of the measurements. The highest relative standard deviations values were found in the blood samples of groups G0 and G4, where they reached up to 20% and 28%, respectively, while in other groups the values were in the range of 13-16%. The variability in blood levels, on the other hand, reflects the differences among individual animals in response to CoQ<sub>10</sub> supplementation. Moreover, blood levels are more time-dependent over a short timescale compared to tissues, as blood is the transport medium of an organism. It was necessary to average the results for blood CoQ<sub>10</sub> levels to obtain a meaningful value as the levels varied so much among individual animals.

The concentration of cholesterol decreased in blood in all groups. The biggest changes were noted in G4 group, after 40 days of feedings chickens with CoQ<sub>10</sub>. This kind of results are expecting according to the data from literature (Honda et al., 2010).

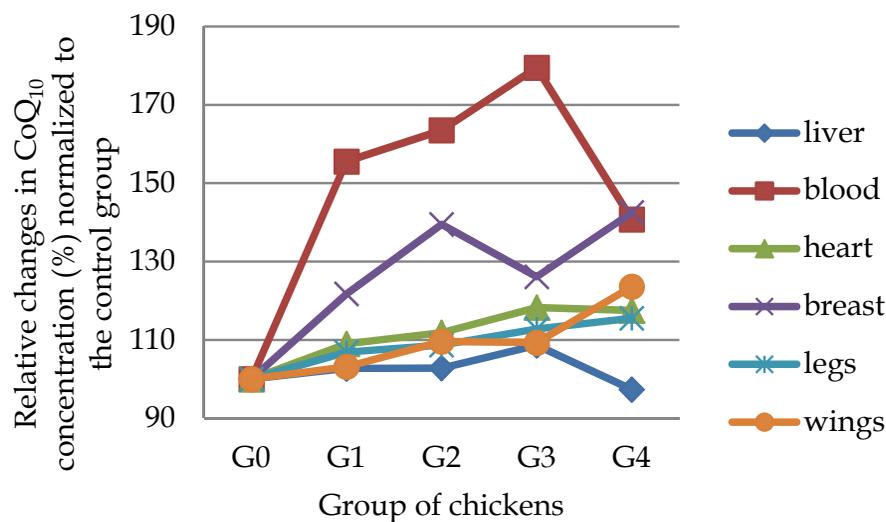


Fig. 6. Relative changes in CoQ<sub>10</sub> concentration during the 40 day feeding period

CoQ<sub>10</sub> is transferred from the blood into various tissue and organs. In this study the concentration of CoQ<sub>10</sub> was examined in heart and muscle tissues (legs, breast and wings). CoQ<sub>10</sub> is present in all tissue and cells but, on a weight basis, in variable amounts. The highest content of CoQ<sub>10</sub> was founded in the most active organs like the heart, kidney, and liver (Ernst & Dallner, 1995).

Statistically, the content of CoQ<sub>10</sub> increased in the hearts of chickens with longer periods of feeding with supplemental CoQ<sub>10</sub>, but only in the range of 7.3% - 11.3%, which is low compared with muscle tissue. Since the supplemented chickens are young organisms, we

therefore assumed that the heart does not require additional CoQ<sub>10</sub>. In heart tissue, concentration of cholesterol was nearly 10 % lower than in the control group.

In the analysis of the muscle tissue, we found that most of the CoQ<sub>10</sub> was found in legs (cca 22 mg/kg), with significantly less in breast (cca 9 mg/kg) and wings (cca 7 mg/kg). The results of our study showed that the added CoQ<sub>10</sub> increased CoQ<sub>10</sub> concentration in all muscle tissue (legs, wings and breast). After 40 days of feeding with feed fortified with CoQ<sub>10</sub> the content of CoQ<sub>10</sub> increased most in breast (45%), followed by wings (25%) and legs (16%). At the same time, concentration of cholesterol in breast, wings and legs are not significantly changed.

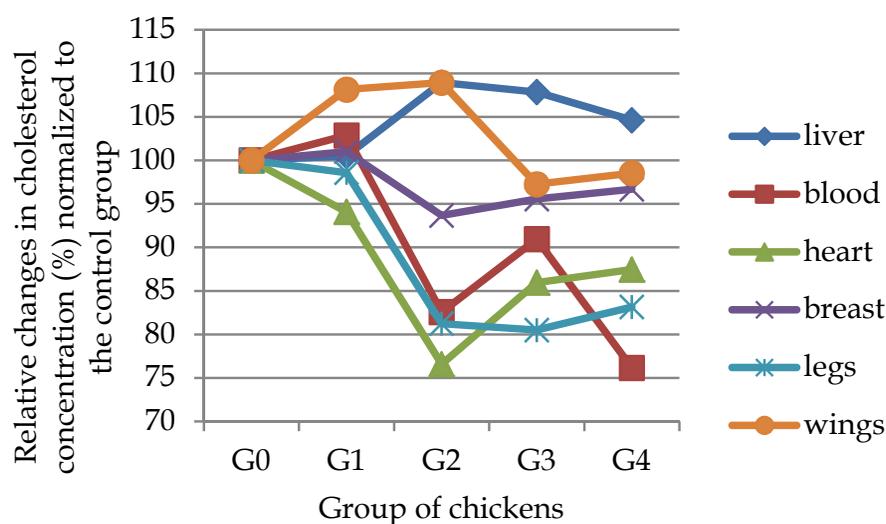


Fig. 7. Relative changes in cholesterol concentration during the 40 day feeding period

The influence of added CoQ<sub>10</sub> on cholesterol concentration was not statistically significant. In the analyzed chicken meat we calculated the QCI (CoQ<sub>10</sub>/cholesterol index) (1), which represents a measure of the improvement in the quality of the meat (Fig.8).

$$QCI = \frac{\text{concentration of CoQ}_{10} \left( \frac{mg}{kg} \right) * 1000}{\text{concentration of cholesterol} \left( \frac{mg}{kg} \right)} \quad (1)$$

The calculated QCI values obtained from the analyses of individual samples from individual animals proved to be a better means of studying CoQ<sub>10</sub> profiles, than the use of absolute CoQ<sub>10</sub> and cholesterol concentrations. The QCI was used, since according to our previous experiments, it is a very reliable indicator of the oxidative status and possible oxidative stresses activated by the food ingredients. We hope that this index will soon receive adequate attention as an informative factor in the evaluation of possible harmful effects of oxidants in foods. The QCI increased in all parts of the chicken, except in the wings where after 10 days of supplemental CoQ<sub>10</sub> feeding, it fell by 9% and after 20 days of supplemental CoQ<sub>10</sub> feeding the value by comparison with the control group hardly

changed. The biggest improvement was achieved in the breast, where, after 20 days of feeding, the QCI relative change index increased by almost 50%.

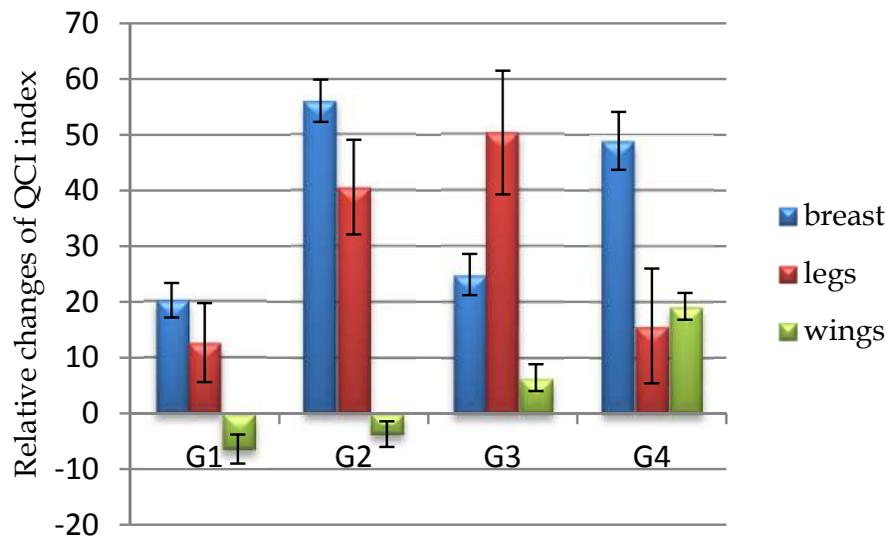


Fig. 8. Relative changes in the QCI ( $QCI = CoQ_{10} \text{ (mg/kg)} / \text{cholesterol (mg/kg)} * 1000$  index) in different chicken muscle tissue in test groups with different periods of feeding with  $CoQ_{10}$

The optimal time of feeding with  $CoQ_{10}$  in comparison with the content of supplemental  $CoQ_{10}$  (mg) during chicken raising was also of interest to this study (Fig. 9). Chicken under prolonged feeding with exogenous  $CoQ_{10}$  received different amount of  $CoQ_{10}$ . Chicken in group G0, G1, G2, G3 and G4 received 0, 50, 100, 150 and 200 mg of  $CoQ_{10}$ , respectively. The results indicated that the highest increase in the concentration of  $CoQ_{10}$  was after 20 days of feeding, when chicken received 100 mg of  $CoQ_{10}$ .

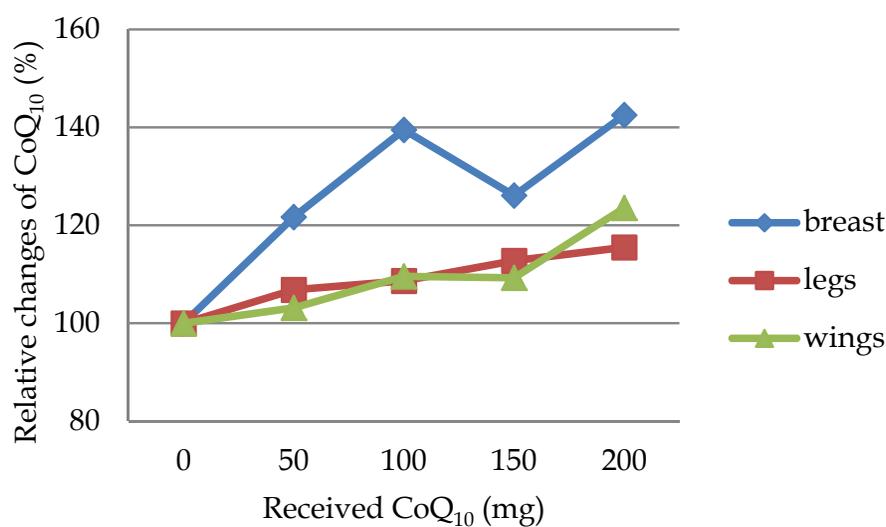


Fig. 9. Relative changes in  $CoQ_{10}$  (%) in chicken meat (legs, wings and breast) in comparison with the content of supplemental  $CoQ_{10}$  (mg) during chicken raising

#### 4.2 Transfer of dietary CoQ<sub>10</sub> into different parts of chicken breast cells

The biggest difference between the concentration of CoQ<sub>10</sub> in the test group G0 and control group G4 was found in the breast tissue (Fig.10). This is the reason why this material was selected for further processing. The selected breast tissue was fractionated into four fractions (Fig. 11) essentially following the procedure from the literature (Casado et al., 1992). Fraction P1 contain mainly nucleus and cell debris, P2 included mitochondria, P3 consist of smaller cell organelles and the remaining S3 fraction was composed of cytosol and small section of disintegrated cell and organelle membranes.

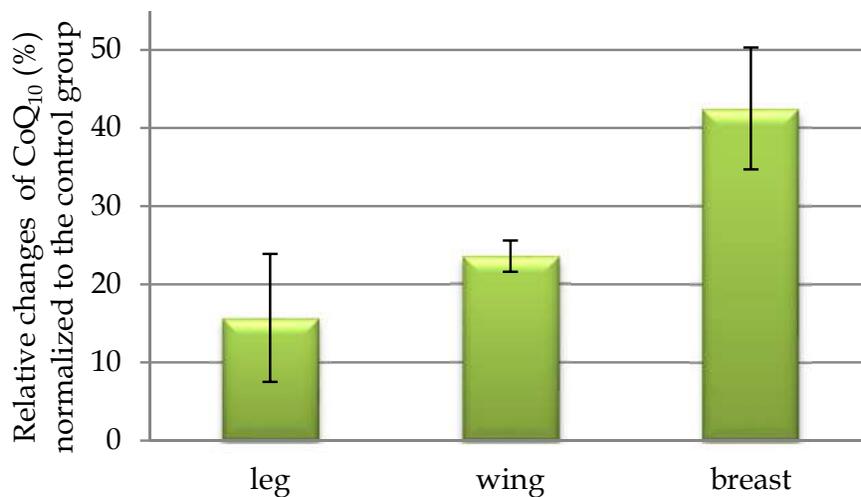


Fig. 10. Relative changes in the concentration of CoQ<sub>10</sub> in several chicken tissue (legs, breast and wings) after 40 days of chicken feeding (G4 group) normalized to the control group (G0 group)

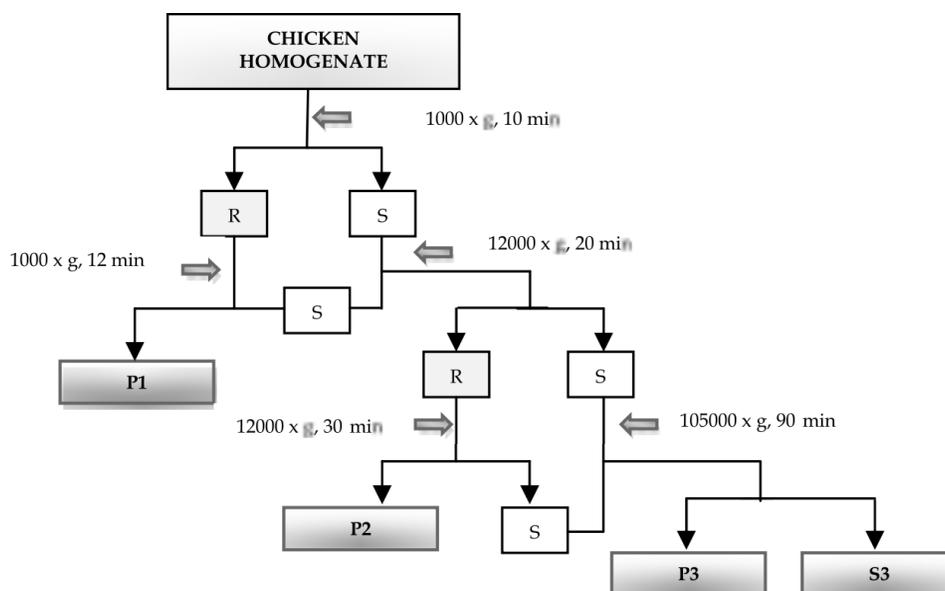


Fig. 11. Fractionation scheme of chicken homogenate (P1 is the nuclear fraction, P2 is the mitochondrial fraction, P3 is the microsomal fraction and S3 is the remaining supernatant)

The concentration of cholesterol differed between the subcellular fractions within each group (P1-P3, S3), but showed practically no difference between groups (G0, G4). At the same time, there were significant differences in the CoQ<sub>10</sub> concentrations with in groups and between fractions in the control and test groups (Table 2). With regard to these results, we decided to normalize the concentrations of CoQ<sub>10</sub> to cholesterol concentrations and not to the protein concentrations that are normally used. The QCI was calculated for all fractions in both groups. The results in Fig. 12 show that the CoQ<sub>10</sub> concentration in the P1 fraction of the G4 group increased by approximately 3 times compared to the control group, meanwhile the increase in the P3 and P4 fraction was merely approximately 30%, but in P2 the amount of CoQ<sub>10</sub> remained unchanged.

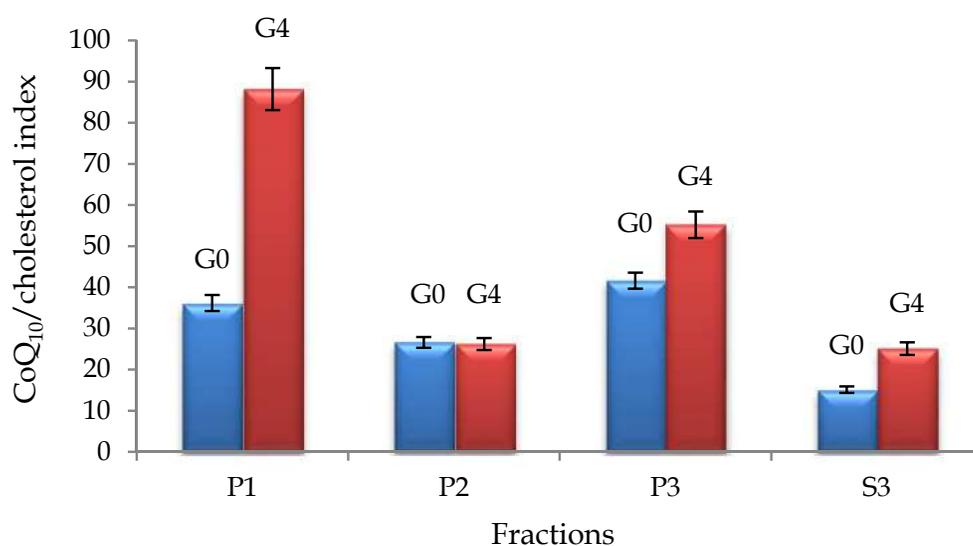


Fig. 12. Values of the QCI analysed by HPLC in different fractions and in the control group G0 and in the G4 test group that received CoQ<sub>10</sub> (5mg/day) for 40 days

The obtained results showed that most of the administered CoQ<sub>10</sub> was located inside the cell membranes. This is seen from the increase in CoQ<sub>10</sub> concentrations in fraction F1, which consisted mostly of the nucleus, partially disintegrated cells, and large cell debris, and cytosol fraction F4 in which small parts of completely disintegrated cell membranes were found. A small increase was also noticed in fraction F3, in which smaller organelles were accumulated. It is very interesting that there is no change in the concentrations of CoQ<sub>10</sub> and cholesterol in the crude mitochondrion fraction (F2). The observed variations in CoQ<sub>10</sub> distribution indicate that supplementary ubiquinone was mainly built into the cell membranes and not into the mitochondria. Theoretically, the maximum increase was expected for the mitochondrial fraction, due to the essential role of CoQ<sub>10</sub> in energy conversion. However, the observed accumulation pattern of CoQ<sub>10</sub> in the nuclear and cytosolic fractions is likely due to other less known or even unknown metabolic functions. In addition, our results are in significant accordance with the concept of the antioxidant action of exogenous supplied CoQ<sub>10</sub>, which is now promoted by Littarru (1994).

In our study, young and healthy animals, who likely have sufficient amounts of endogenous CoQ<sub>10</sub>, were used and this could be the main reason why the exogenous addition was built into the cell membranes and not into the mitochondrion inner membranes.

### 4.3 Poultry products with an increased content of CoQ<sub>10</sub>

The majority of consumers refuse to eat meat with higher levels of fat, due largely to the possible association between high levels of cholesterol and heart disease. Poultry meat and poultry products are widely consumed due to their lower content of cholesterol than other meat, faster digestibility, and price accessibility. Meat is a rich source of essential amino acids, minerals, some vitamins, and long chain polyunsaturated fatty acids. Thus, a moderate intake of meat is important part of balanced diet.

Supplementary dietary CoQ<sub>10</sub> present in food or taken as a dietary supplement is widely consumed because of its beneficial effect on human health. Supplementation with CoQ<sub>10</sub> has been shown to improve the resistance of LDL particles to oxidation and to prevent atherogenicity (Hanaki et al., 1993; Witting et al., 2000).

The aim of our work was to prepare food with an increased content of CoQ<sub>10</sub> from chicken fed with supplemental CoQ<sub>10</sub>. This procedure of preparing food with supplemental CoQ<sub>10</sub> by direct feeding provided also health benefits to the chicken.

#### 4.3.1 Animals and experimental design

Two groups of 1-day-old ROSS 308 male chickens were obtained from the regular hatching process in a poultry hatchery. The control group of animals (36600 chickens) was fed plain feed for the whole period, while the study group (37000 chickens) received the last 20 day feed fortified with water-soluble CoQ<sub>10</sub>-βCD. The shortened feeding period was selected because the laboratory experiment showed that a major increase in the concentration of CoQ<sub>10</sub> in meat tissue was provoked after the twenty-day administration period. The concentration of CoQ<sub>10</sub> in feed was calculated so that each animal received an average of approximately 5 mg of CoQ<sub>10</sub> per day. A set of poultry food products (breaded chicken wings, breaded chicken drumsticks, breaded chicken fillet, chicken nuggets, extra chicken sausages, and chicken liver pate) was prepared from the meat and organs (liver, heart) of the test animals according to usual industrial procedures. Concentrations of CoQ<sub>10</sub> and cholesterol were evaluated using two reliable analytical procedures, quantitative HPLC-MS and semi-quantitative TLC.

#### 4.3.2 QCI in poultry products

The content of CoQ<sub>10</sub> increased as expected in all chicken products, while the concentration of cholesterol were only slightly reduced. The content of CoQ<sub>10</sub> concentration in different chicken meat and their products are shown in Fig. 13. The results show that the poultry pates contain the highest value of CoQ<sub>10</sub>. The concentration of CoQ<sub>10</sub> increased in all poultry products according to the increased value of CoQ<sub>10</sub> in the fortified chicken meat.

From the obtained results we calculated the QCI to determine the improvement in meat quality. The relative changes in the QCI concentration normalized to the control group are presented in Fig. 14. The results show the improvement for the fortified poultry products. The highest increase in the QCI was obtained in fortified chicken nuggets (220%) and in breaded chicken wings (206%).

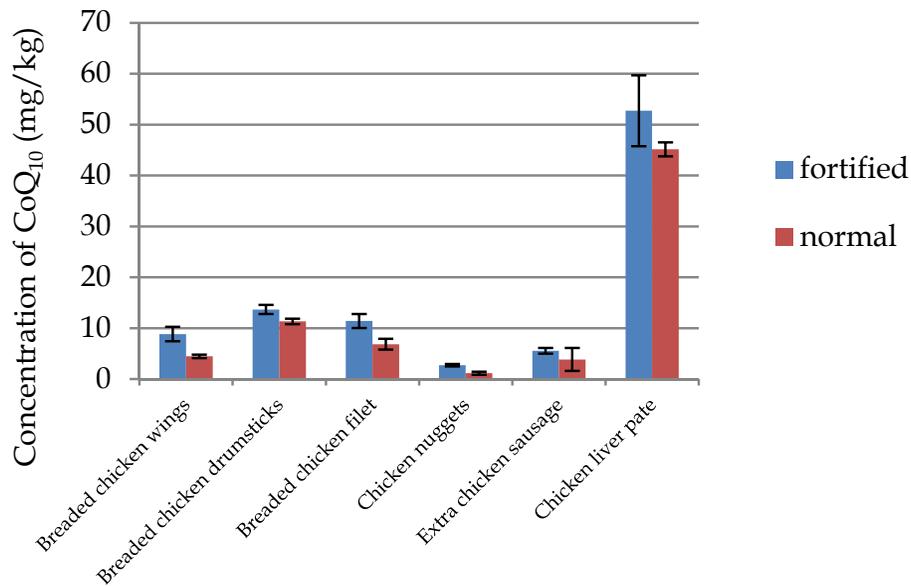


Fig. 13. CoQ<sub>10</sub> concentration (mg/kg) in different meat (breast, wings and legs) and in different poultry products (breaded chicken wings, breaded chicken drumsticks, breaded chicken fillet, chicken nuggets, extra chicken sausages, and chicken liver pate)

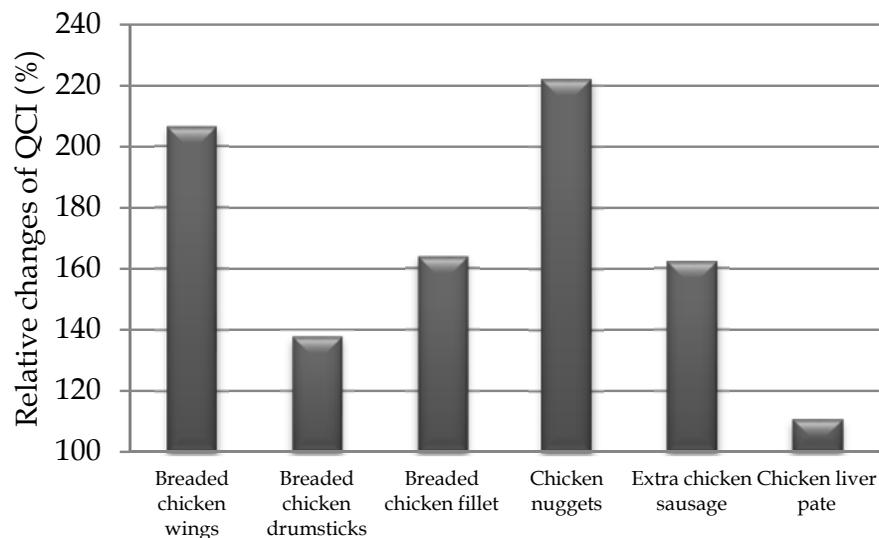


Fig. 14. Relative changes in the QCI (%) in different poultry products normalized to control groups

The CoQ<sub>10</sub> content in people's diets was determined to be 3-6 mg per day, primarily derived from meat. The fortified chicken liver pate contains an average of 52.7 mg CoQ<sub>10</sub>/kg sample, meaning that if the consumer ingests 100 g of the product, he/she gets approximately 5.3 mg of CoQ<sub>10</sub>. Breaded chicken wings, breaded chicken drumsticks and breaded chicken fillets contain large amounts of CoQ<sub>10</sub>, but these are products that require heating prior to ingestion. Weber et al. (1997) studied the loss of CoQ<sub>10</sub> during food preparation. The results showed that frying reduces CoQ<sub>10</sub> levels by 14-32%, while boiling does not reduce CoQ<sub>10</sub> levels.

Food that contains CoQ<sub>10</sub>, is a more complex matrix than capsules. The absorption of the various components of food from the gastrointestinal tract is one of the major determinants of bioavailability. Literature data indicate that intestinal absorption of food accelerates CoQ<sub>10</sub> absorption (Ochiai et al., 2007). It is known that foods containing fat promote the excretion of bile acids, which form micelles water-insoluble components and thus increase the possibility of absorption through the gastrointestinal tract. Fear that the enriched products will exceed the current recommended daily dose, which should amount to 30 mg/day, is not grounded. In fact, these fortified products would increase the intake of CoQ<sub>10</sub>, which numerous studies indicate to be positive.

## 5. Conclusions

Coenzyme Q<sub>10</sub> is a key component in the inner mitochondrial membrane, where it plays important role in oxidative phosphorylation. It is also present in other subcellular fractions and in plasma lipoproteins, where it has antioxidant properties. CoQ<sub>10</sub> has also been recognized to have an effect on gene expression. These three functions are important for its use in clinical practice and as a food supplement. A large number of clinical studies indicate that dietary CoQ<sub>10</sub> administration has beneficial effects, particularly in cardiomyopathies, as well as degenerative muscle and neurodegenerative diseases.

The bioavailability of CoQ<sub>10</sub> is relatively low due to its lipophilic nature and relatively high molecular mass. Increasing the availability of CoQ<sub>10</sub> in aqueous medium could consequently also increase its bioavailability, which can be achieved by complexing CoQ<sub>10</sub> with  $\beta$ -CD.

The aim of our work was to study the effect of added CoQ<sub>10</sub> in water soluble form in chicken feed on broiler chicken and to prepare functional chicken products with biologically incorporated CoQ<sub>10</sub>. The weighing of chicken showed that the greatest growth was achieved in chicken groups where the chickens were fed with added CoQ<sub>10</sub> for 40 days.

The results of our study showed that added CoQ<sub>10</sub> increased CoQ<sub>10</sub> concentration in plasma, heart, and in chicken meat (breast, legs, wings). The added CoQ<sub>10</sub> decreased cholesterol concentration in the heart and in plasma. The highest increase in CoQ<sub>10</sub> concentration was observed in chicken breast cells after 40 days of feeding with CoQ<sub>10</sub> added in feed. Fractionation of chicken breast cells showed that the added CoQ<sub>10</sub> was mainly incorporated into cell membranes and not into mitochondria. This confirmed our hypothesis that CoQ<sub>10</sub> added in chicken tissues acts mostly as an antioxidant.

In the industrial experiment, chickens were fed with CoQ<sub>10</sub> added in feed for the last 20 days before slaughtering and the functional products were prepared from meat fortified with CoQ<sub>10</sub>. As expected, the content of CoQ<sub>10</sub> increased in all chicken products especially in breaded chicken fillet, while nearly in all meat tissues concentration of cholesterol was slightly reduced which increased the QCI. We presume that, QCI can be used as useful indicator in meat nutritional quality which also indicates better growing conditions for chickens regarding to oxidative stress. Fortified chicken products can have a positive effect on consumer's health. Accumulated CoQ<sub>10</sub> together with simultaneously absorbed fats can protect non-saturated fats from uncontrolled oxidation and consequently reduce the risk of different diseases.

In the next research period, we will deal with the importance of antioxidant network in preventing of oxidative stress. We also expect that the obtained results will help us to get

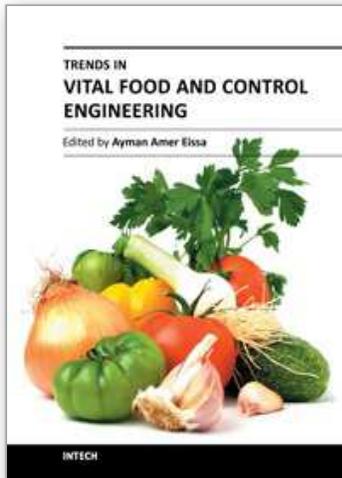
enough information to design new functional food and feed additives and to provide better breeding production of chickens.

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