THE EFFECTS OF DIETARY VITAMIN E SUPPLEMENTATION ON SWINE MEAT AND COOKED HAM CHOLESTEROL AND CHOLESTEROL OXIDES

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Background

The meat industrial storage and processing procedures affect lipid and cholesterol oxidation. Heat treatment, shredding, mincing, storing and sodium chloride addition are available for oxidative catalysis (Kanner, 1994). Cholesterol oxidation may proceed by the same mechanisms for lipid oxidation, producing a wide variety of cholesterol oxide (Kim & Nawar, 1994). Dietary oxysterols may substantially increase the atherogenicity of lipoproteins and the oxidation of triglyceride-rich lipoprotein (Vine *et al.*,1998). They also may cause cytotoxicity, angiotoxicity, mutagenicity and even carcinogenicity (Smith, 1987). One way to increase the oxidative stability of lipids and cholesterol in food is to increase the amount of natural antioxidants such as α-tocoppherol (vitamin E) or β-carotene in the diet. Feeding diets supplemented with vitamin E applied to animals like chickens, cows, and pigs resulted in vitamin accumulation in the animal muscle and better oxidative stability under prooxidative condition such as storage and cooking (DeWine & Dirinck, 1997).

Objectives

Demonstrate the protective effect of feeding diets supplemented with vitamin E on cholesterol oxides in the cooked ham, an important processed meat consumed all over the world.

Methods

Sixteen crossbred pigs (*L.W X L P*), eight barrows and eight gilts were randomly alloted to one of the four treatment groups: 1) control diet containing no supplementary vitamin E, 2) diet formulated to contain 100 mg of vitamin E/kg diet, 3) 200 mg of vitamin E/kg diet, 4) 400 mg of vitamin E/kg diet. The diets were supplemented with vitamin E in the form of α-tocopherol (Rovimix 50%®, Hoffmann-LaRoche, nutley, NJ – USA). The feeding period was completed in 116 days, and, then, the pigs were immediately slaughtered. After the chilling period, the *biceps femoris* muscles were removed from the carcass to produce the cooked hams. Before the cooked ham processing, the *biceps femoris* samples were taken and stored at -20ºC for the analysis. The cooked ham was produced in an industrial unit, and were stored at at 5ºC during 2 months to be analysed. The *biceps femoris* samples were thawed and homogenated with blender. The extration and determination of total lipids were undertaken according to Folch, Less and Stanley's method (1957). The cholesterol and cholesterol oxide were determined simultaneously by High Performance Liquid Chromatography according to Sander *et al* method (1989) modified by Baggio and Bragagnolo (2000). The cholesterol and cholesterol oxides of cooked ham were analysed the same way described above at 0, 30 and 60 storing days. The statistical signicance of the difference between the cholesterol levels in *biceps femoris muscle* and cooked ham was determinad by ANOVA. The difference significance among the averages was determined by Tukey test. The statistical analysis of the cholesterol and cholesterol oxides from cooked ham among the treatments and sex during 60 days period (0, 30 and 60 days) was tested in a split-plot design (Gomes, 1985). All data were analyzed using the General Linear Model procedure of SAS(1999).

Results and Discussions

As the vitamin E levels in the diet were increased, a reduction in cholesterol contents occurred, reaching 30% in the supplementation levels of 400 mg of vitamin E/kg diet. As far as we know information on the reduction of the cholesterol levels in swines with the vitamin E ingestion has not been found in literature until the present. A significant difference ($P < 0.05$) among the sexes were observed, with the barrows samples presenting an average of 41.46 ± 0.42 mg/100 g, superior to the ones of the gilts, that is, 38.16 ± 0.40 mg/100g. The differences observed in the cholesterol levels among the barrows and gilts can be attributed to the hormone factors (Wenk *et al.*, 1980), since the animals had received the same diet and had had the same treatment. Rowe *et al.* (1997) observed the average 47.39 ± 3.24 mg/100g for the cholesterol content in swine ham. Therefore, they are levels very close to those observed in the present work, despite the use of different analysis methodology, and the sample submission to cook in boiling. Baggio and Bragagnolo (2000) using the same methodology of this study observed the following cholesterol contents: 46.0 ± 5.0; 35.0 ± 2.0; 27.0 ± 3.0 e 81.0 ± 6.0 mg/100g in the turkey's wing, thigh, chest and skin, respectively. The cholesterol levels observed for the turkey's wing are close to those obtained in the ham control samples. During the cooked ham processing a significant reduction ($P < 0.05$) in cholesterol contents occurred, in all the treatments and sexes, comparing the results obtained in the ham and cooked ham, immediately after the processing. We suppose that this relative reduction is probably due to the ham mass increase with the injection of the cure solution, since in zero time storage cholesterol oxides were not observed in the samples of cooked ham. During the storing period, a decrease in cholesterol contents in cooked ham occurred (Figure 1), but more evident in treatment 1 (control), reducing to at the same time that increasing the levels of vitamin E in the diet. The reduction in cholesterol contents during the storage of cooked ham, clearly observed in treatments 1 and 2, was followed by the increase in cholesterol oxide contents (Figure 2), confirming their origin. The cholesterol levels for treatment 4 samples remained practically constant with supplementation levels of 400 mg of vitamin E/kg feed. In ham and cooked ham samples, in zero time (initial) storage, cholesterol oxides had not been detected in the treatments or sex. After 30 storing days cholesterol oxides had been detected in the cooked ham samples (treatments 1 and 2). The cholesterol oxide found was cholesta-4,6-dien-3-one (dieno), in concentrations varying from 2.56 ± 0.08 to 4.18 ± 0.11 μg/g; and 20 α-hydroxycholesterol (20R-OH), in concentrations varying from 2.22 ± 0.07 to 3.15 ± 0.11 μg/g. During the 60 storing days, a substantial increase in the total cholesterol oxide, in the cooked samples ham of treatments 1 and 2, occurred. This increase is mainly due to the presence of 24-ketocholesterol (cholest-5-en-24-one), in concentrations varying from 34.15 ± 1.48 to 60.11 ± 2.61 μg/g. Also, 25-hydroxycholesterol was found in treatments 1 and 2 samples, however, in small concentrations, varying from 4.18 ± 0.13 to 6.33 ± 0.19 μg/g. In treatment 3 samples cholesta-4,6-dien-3-one was detected in the concentrations varying from 2.66 ± 0.07 to 3.22 ± 0.08 μg/g, and 20α-hydroxycholesterol in the concentrations varying from 2.60 ± 0.08 to 2.99 ± 0.09 μg/g, presenting a behavior similar to treatment 2 with 30 storing days. No cholesterol oxide was observed in the treatment 4 samples. Monahan *et al.* (1992) also observed a significant reduction in cholesterol oxide contents, in the swine meat samples with supplementation of 200 mg of vitamin E/kg, when stored in cold place after cooking. The cholesterol oxide cholesta-4,6-dien-3-one has been observed in different food products, such as bovine and swine fat (Chen *et al.*, 1994); bovine meat (Vajdi & Nawar, 1979), chicken meat (Hwang & Maerker, 1993), with values varying from 0.36 to 5.9 μg/g, depending on the processing and storage. The cholesterol oxide 20 α-hydroxycholesterol was reported

in swine and bovine meat (Pie *et al.*, 1991), salami and parmesan cheese (Schmarr *et al.*, 1983), in concentrations varying from 0.14 to 10.88 µg/g. Considering the turkey meat, the cholesta-4,6-dien-3-one and 20 α-hydroxycholesterol were also detected in concentrations from 0.33 ± 0.03 to 7.65 ± 0.87 µg/g in the samples obtained in the market (Baggio & Bragagnolo, 2000). 25-hydroxycholesterol is considered the most cytotoxic cholesterol oxide, it was found in swine meat and poultry (Finochiaro & Richardson, 1983), in Milano salami and mortadella (Novelli *et al.*, 1998), in concentrations varying from 0.42 to 15.82 µg/g. References on 24-ketocholesterol in food have not been found, however, their presence in cooked ham samples were confirmed in HPLC and GC-MS, with 97% probability.

Conclusion

Levels of 400 mg of vitamin E/kg feed supplied during 116 days before slaughtering reduces 30% of cholesterol in ham samples. The same feeding period, 200 mg supplemented levels of vitamin E/kg feed or more maintained the total cholesterol oxide values below 10 µg/g (superior limit for health) in the cooked ham during 60 cold storing days.

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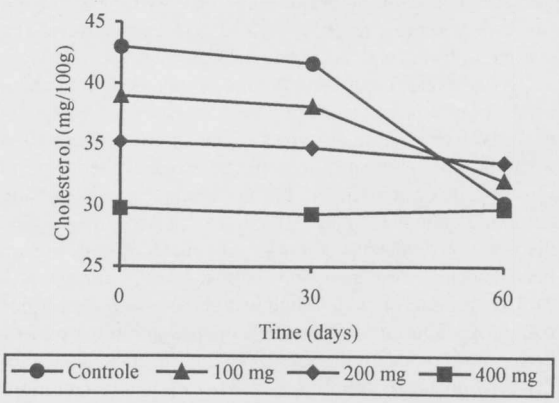

Figure 1. Vitamin E effects on cholesterol

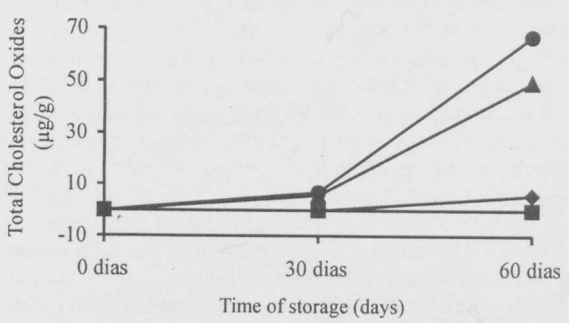

Figure 2. Vitamin E effects on oxysterols