## EFFECT OF DIETARY IRON AND COPPER REMOVAL ON CHOLESTEROL AND LIPID OXIDATIVE STABILITY OF BROILER MEAT

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### Background

Lipid and cholesterol oxidation are oxidative processes affecting meat quality and safety (Gray *et al.*, 1996). Secondary products like aldehydes and oxysterols are thought to be involved in the etiology of cancer and cardiovascular disease (Ames, 1983; Pearson *et al.*, 1983). Oxysterols and aldehydes result from the decomposition of cholesterol and lipid hydroperoxides and this reaction can be catalyzed by transition metals like iron and copper. These reactions can take place in the muscle tissue under prooxidative conditions like cooking and storage of meat (Kanner, 1993). The generation of oxidation products in meat decreases its safety since oxysterols have been shown to be readily absorbed from the diet and possess adverse biological activities (Smith and Johnson, 1989; Emanuel *et al.*, 1991). In muscle foods, the non-haeme iron (NHI) pool includes the storage proteins like ferritin and haemosiderin and a low molecular weight iron fraction (Kanner, 1993). This free iron is in the chelated form and is thought to catalyze lipid peroxidation (Kanner *et al.*, 1988; Decker *et al.*, 1993). Unlike iron, the amount of free copper ions in muscle foods is very low and mostly chelated to carnosine which prevents them to catalyze lipid peroxidation (Chan and Decker, 1994).

### Objectives

Dietary iron have been shown to influence NHI in pork muscle tissues (Miller *et al.*, 1994a). Therefore, this study was designed to investigate the effect of a reduced ingestion of the prooxidants iron and copper on the cholesterol and lipid oxidative stability of broiler meat. It was hypothesized that a reduced ingestion of trace elements would decrease the cellular iron and copper stores and consequently would reduce their catalytic effect on lipid peroxidation.

### Methods

504 female broiler chicks of the Ross strain were used. They were placed in 48 flat-deck cages, 1 square meter each, in the flat-deck cages room. The chicks were raised according to routine practices in terms of light and temperature. Maize-soybean based diets containing sunflower oil, were fed to broiler chicks for six weeks. One group of chicks was supplemented with 9 mg of Cu and 85 mg of Fe (normal levels) throughout the growing period (control group; for composition see **Table 1**). Another group was supplemented with Cu only, during the last three weeks. Another group was supplemented with Fe only, during the last three weeks. Finally, one group was unsupplemented during the last three weeks. Oxysterols, α-tocopherol, the glutathione peroxidase activity and the TBARS were determined in raw thigh meat. Thighs were also cooked in polyethylene bags. Samples were placed in a water bath at 85°C, until inside temperature reached 80°C (50 minutes). Samples were immediately processed for oxysterols and TBARS. Oxysterols were determined by using the method of García Regueiro and Maraschiello (1997). α-Tocopherol analysis was performed by using the procedure of Maraschiello and García Regueiro (1998). The procedure used for TBARS analysis was a modification of the method of Botsoglou *et al.* (1994). The glutathione peroxidase (GSHPx) activity was determined by using the assay developped by De Vore and Greene (1982).

### Results and Discussion

It seems that the mechanisms linking the dietary ingestion of trace elements and their cellular stores are quite complex. Minimal cholesterol oxide levels (0.10-0.20 µg/g) were produced in raw meat (**Table 2**). Regarding the TBARS, the results (0.5-0.6 µg MDA equivalents/g tissue) agreed with those reported in the literature for chicken meat (Galvin *et al.*, 1993). The activity of the GSHPx did not significantly vary as a consequence of the prooxidants removal (**Table 2**). The tissue α-tocopherol levels were similar for each dietary groups (± 5 µg/g, see **Table 2**). Nonetheless, the TBARS values and the GSHPx activity of the meat from the broilers deprived of either iron or copper tended to indicate a higher oxidative stress. These differences were accentuated when the meat was cooked (**Tables 2** and **3**). Significant differences were obtained for the TBARS values but not for oxysterols, although higher values were obtained for the groups deprived of either iron or copper (**Table 3**). The reasons for this phenomenon remained unclear. It is possible that the withdrawal of the iron or the copper from the diet could have perturbed some metabolic pathways like those modulating the lipid composition of the muscle tissue. Dietary copper has been found to influence the lipid metabolism and the fatty acid composition of porcine depot fats (Elliot and Bowland, 1968). Oxysterol levels increased 10-fold when meat was cooked, as already reported (Maraschiello *et al.*, 1998). This increase in the oxysterol concentrations was accompanied by an increase in the TBARS (**Tables 2** and **3**).

### Conclusions

It can be concluded that the removal of iron and copper from the animal diet did not affect significantly the oxidative stability of raw meat. Similar results were reported by Miller *et al.* in raw and cooked meat when dietary iron intake was reduced in swine (Miller *et al.*, 1994b). Kanner *et al.*

evidenced an increased oxidative stability during storage, in meat from turkey deprived of iron (Kanner *et al.*, 1990). Nonetheless, they did not detect any differences in the fresh meat (Kanner *et al.*, 1990). Our results suggest further research in order to demonstrate a possible effect of the reduced iron and copper ingestion on the oxidative stability of lipids and cholesterol in muscle meat, since prooxidizing conditions like cooking evidenced some differences between the dietary treatments.

**Acknowledgements**

This work was carried out within the framework of the European concerted action DIET-OX (AIR protject No. PL 921577) : "Dietary treatment and oxidative stability of muscles and meat products. Nutritive value, sensory quality and safety". C. Maraschiello was holder of an EC FAIR grant (FAIR-CT96-5081).

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**TABLE 1. Composition of the basal diet.**

| Ingredients | % |
|---|---|
| Maize | 50.56 |
| Soybean meal | 39.58 |
| **Sunflower oil** | 6.00 |
| Calcium carbonate | 1.00 |
| Dicalcium phosphate | 2.00 |
| Salt | 0.40 |
| DL-methionine | 0.16 |
| Vitamins and minerals[a] | 0.40 |

[a] One kg of feed contained : Vitamin A : 12000 UI ; Vitamin D3 : 2400 UI ; **Vitamin E : 20 mg** ; Vitamin K3 : 2 mg ; Vitamin B1 : 2 mg ; Vitamin B2 : 5 mg ; Vitamin B6 : 3.5 mg ; Vitamin B12 : 15µg ; Folic acid : 0.6 mg ; Biotin : 200 µg ; Calcium Pantothenate : 15 mg ; Nicotinic acid : 30 mg ; Mn : 332 mg ; Zn : 50 mg ; I : 1.19 mg ; **Fe : 85 mg** ; **Cu : 9 mg** ; **Se : 0.15 mg**.

**TABLE 2. Prooxidant experiments[1]. Oxysterols, TBARS, α-tocopherol and GSHPx activity in raw meat.**

| Parameter | Control group | Iron removed | Copper removed | Iron and copper removed | SE |
|---|---|---|---|---|---|
| Total amount of oxysterols[2] | 0.20[a] | 0.15[a] | 0.18[a] | 0.12[a] | 0.03 |
| TBARS | 0.48[a] | 0.62[a] | 0.62[a] | 0.48[a] | 0.06 |
| α-Tocopherol | 4.80[a] | 4.40[a] | 4.87[a] | 4.79[a] | 0.46 |
| GSHPx activity | 8.08[a] | 8.53[a] | 9.29[a] | 8.38[a] | 0.59 |

[1]Results are expressed as Least Square means (LSmeans) values in µg/g tissue for oxysterols. TBARS in µg malonaldehyde/g muscle tissue; SeGSHPx activity in nmol NADPH / min / mg of protein; α-tocopherol in µg/g muscle tissue. SE, standard error of the LSmeans. n=8 for each experiment.
[2]The analysis includes the determination of 7α- and 7β-hydroxycholesterol; β- and α-epoxycholesterol; 20α-hydroxycholesterol; cholestanetriol; 25-hydroxycholesterol; 7-ketocholesterol.
[a,b]Different letters noting significant differences between columns ($p<0.05$).

**TABLE 3. Prooxidant experiments[1]. Oxysterols and TBARS in cooked meat.**

| Parameter | Control group | Iron removed | Copper removed | Iron and copper removed | SE |
|---|---|---|---|---|---|
| Total amount of oxysterols | 1.46[a] | 2.22[a] | 1.99[a] | 1.52[a] | 0.30 |
| TBARS | 6.38[a] | 9.21[b] | 10.69[b] | 8.30[a,b] | 0.88 |

[1] See Table 2.