# LIPID AND CHOLESTEROL OXIDATION IN α-TOCOPHEROL-SUPPLEMENTED CHICKEN AS AFFECTED BY SALT AND CARNOSINE

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## INTRODUCTION

Lipid oxidation is an important factor influencing the quality of meats. In addition to contributing to undesirable changes in the sensory characteristics of meats, oxidation also results in the formation of a number of compounds, including cholesterol oxidation products (COPs), which may adversely affect human health. The oxidation of lipids is of particular importance in processed meats, as these are exposed to pro-oxidizing preparation methods such as mincing and cooking. Additives, particularly salt, can also exhibit pro-oxidant activity. Oxidation is influenced by free radicals and pro-oxidants present in the lipid and aqueous phases of the muscle cell. Therefore, both lipid and water-soluble antioxidants would be expected to play a role in the control of lipid oxidation in meats. α-Tocopherol is a highly effective lipid-soluble antioxidant in meats, particularly during processing and storage. Dietary supplementation with α-tocopherol increases muscle membrane α-tocopherol levels, and improves the stability of neighbouring unsaturated phospholipids. Supplementation also inhibits the formation of COPs during storage. The post-slaughter addition of antioxidants has also been used to improve lipid stability. Carnosine, a skeletal muscle dipeptide, has been shown to reduce lipid oxidation and improve colour stability when added to minced meat (Decker & Crum, 1991). It is believed to play a multifunctional role in removing water-soluble pro-oxidants, and has been shown to scavenge free radicals (Chan *et al.*, 1994) and chelate metal ions such as iron and copper (Decker *et al.*, 1992). The effects of carnosine on lipid and cholesterol oxidation during storage, when used in combination with other antioxidants is unclear. The purpose of this experiment was to determine the effect of the post-slaughter addition of carnosine, in combination with dietary α-tocopherol supplementation, on lipid oxidation in processed chicken.

## METHODS

Cobb 500 broiler chicks were randomly divided into 2 groups and were fed diets supplemented with 30 or 200mg α-tocopheryl acetate/kg. Birds were slaughtered after 6 weeks. Thigh muscle was removed and stored at -20°C until required. Prior to the storage stability study, muscle was thawed at 4°C. Muscle was trimmed of extramuscular fat, minced and mixed with salt (1%) and carnosine (1.5%) dissolved in water (5%). Patties (~50g) were prepared with no salt or carnosine (control), salt only, carnosine only, or salt plus carnosine, and were cooked in a conventional oven, to an internal temperature of 75°C. Following cooling, samples were placed in polyethylene bags and stored under fluorescent light, at 4°C for 7 days. Samples were assessed for lipid and cholesterol oxidation on days 0, 2, 4 and 7.

Samples for lipid oxidation analysis were prepared using the distillation procedure of Ke *et al.* (1977). Distillates were reacted with thiobarbituric acid (TBA) according to the method of Tarladgis *et al.* (1960). The red chromogen formed was subjected to derivative spectral analysis, using a modification of the procedure of Espinosa-Mansilla *et al.* (1993). Results were reported as MDA-TBA values and were expressed as nmol malonaldehye (MDA)/g meat.

Total lipids for COPs analysis were extracted from muscle by the method of Marmer & Maxwell (1981). COPs were separated from cholesterol and other lipids by the procedure of Park & Addis (1985) and were determined by a GC method.

Lipid and cholesterol oxidation data were subjected to two-way ANOVA, with variation attributed to salt and carnosine. Within basal and supplemental groups, all pairs of means were compared by the method of least significant difference (Snedecor & Cochran, 1967).

## RESULTS AND DISCUSSION

Dietary α-tocopherol supplementation improved the storage stability of processed meat. When corresponding basal and supplemental groups were compared, MDA-TBA values were 8.5-13.5-fold lower in supplemental groups during the course of the study.

In basal meat, MDA-TBA values were significantly ($p<0.01$) higher in samples with added salt than in control samples at all time points (Table 1). The addition of carnosine reduced TBA-MDA values significantly ($P<0.001$) compared to control values. When treatments with salt and salt plus carnosine were compared, no differences were observed on days 0 and 2. On days 4 and 7, MDA-TBA values were significantly ($P<0.001$) lower in samples with carnosine.

In supplemental meat, MDA-TBA values were significantly ($P<0.05$) higher in samples with salt than in the control. The addition of carnosine resulted in lower MDA-TBA values than in the control. Lipid oxidation was also lower in samples with salt plus carnosine than in samples with salt only.

α-Tocopherol supplementation also resulted in lower levels of COPs formed during storage. The effects of salt and carnosine on COPs formation during storage were similar to their effects on lipid oxidation (Table 2). In basal meat, the addition of salt had no effect on COPs on day 0, but resulted in the formation of significantly ($P<0.05$) higher levels of COPs than in the control on all subsequent days. Carnosine significantly ($P<0.05$) reduced COPs levels on all days. COPs were also significantly ($P<0.05$) lower in samples with salt plus carnosine than in samples with salt only.

In supplemental meat, salt significantly ($P<0.05$) increased COPs levels on days 4 and 7. Carnosine significantly ($P<0.05$) reduced the levels of COPs formed on all days. Cops levels were also significantly ($P<0.05$) lower in samples with salt plus carnosine than in samples with salt only.

## CONCLUSIONS

Salt accelerated lipid and cholesterol oxidation in processed chicken during refrigerated storage. Carnosine inhibited lipid oxidation and COPs formation, and also protected meat from the prooxidizing effects of salt. Dietary α-tocopherol supplementation reduced lipid and cholesterol oxidation. Overall, dietary α-tocopherol supplementation plus carnosine provided the greatest antioxidant protection against salt. These results suggest that a combination of dietary α-tocopherol supplementation and post-slaughter carnosine addition may provide the best means of improving lipid stability in processed meats.

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Table 1. Effect of salt and carnosine on lipid oxidation in cooked minced thigh meat from broilers fed diets containing basal (30mg/kg) or supplemental (200mg/kg) levels of α-tocopheryl acetate.

| α-Tocopheryl acetate | Treatment[1] | Days at 4°C 0 | 2 | 4 | 7 |
|---|---|---|---|---|---|
| | | MDA-TBA (nmol/g meat) | | | |
| Basal | Control | 4.23±0.13[a] | 61.24±4.38[a] | 82.42±3.91[a] | 121.31±2.82[a] |
| | Salt | 7.52±0.89[b] | 78.36±6.28[b] | 93.23±4.10[b] | 135.29±4.16[b] |
| | Carnosine | ND | 3.58±0.17[c] | 5.91±0.81[c] | 7.22±0.50[c] |
| | Salt + carnosine | ND | 4.01±0.07[c] | 6.92±0.23[d] | 9.74±0.95[d] |
| | Effect of salt | * | * | * | ** |
| | Effect of carnosine | ** | ** | ** | ** |
| Supplemental | Control | ND | 4.64±0.56[a] | 6.10±0.15[a] | 14.32±1.94[a] |
| | Salt | ND | 5.06±0.32[a] | 7.91±0.19[b] | 17.58±2.16[b] |
| | Carnosine | ND | ND | ND | ND |
| | Salt + carnosine | ND | ND | ND | 1.37±0.08[c] |
| | Effect of salt | NS | NS | ** | ** |
| | Effect of carnosine | NS | ** | ** | ** |

1 Salt, 1%; Carnosine, 1.5%.
Values are means ± SEM of 4 analyses performed in duplicate.
ND = Not detected.
a-d Within basal and supplemental treatments, means in the same column bearing different superscripts are significantly different (ANOVA, LSD): *P<0.01, **P<0.001, NS = not significant.

Table 2. Effect of salt and carnosine on the formation of cholesterol oxidation products (COPs) in cooked minced thigh meat from broilers fed diets containing basal (30mg/kg) or supplemental (200mg/kg) levels of α-tocopheryl acetate.

| α-Tocopheryl acetate | Treatment[1] | Days at 4°C 0 | 2 | 4 | 7 |
|---|---|---|---|---|---|
| | | Total COPs[1] (μg/g meat) | | | |
| Basal | Control | 0.75±0.11[a] | 4.29±0.34[a] | 6.38±0.28[a] | 13.44±0.42[a] |
| | Salt | 0.85±0.11[a] | 4.96±0.21[b] | 6.91±0.17[b] | 14.85±0.55[b] |
| | Carnosine | 0.38±0.06[b] | 2.76±0.25[c] | 3.97±0.25[c] | 10.34±0.31[c] |
| | Salt + carnosine | 0.35±0.05[b] | 2.88±0.19[c] | 4.85±0.23[d] | 11.91±0.29[d] |
| | Effect of salt | NS | * | * | ** |
| | Effect of carnosine | * | * | ** | ** |
| Supplemental | Control | 0.42±0.06[a] | 3.26±0.20[a] | 4.58±0.12[a] | 14.53±0.39[a] |
| | Salt | 0.49±0.03[a] | 4.07±0.31[b] | 5.62±0.30[b] | 12.60±0.49[b] |
| | Carnosine | ND | 1.78±0.15[c] | 2.75±0.27[c] | 6.62±0.26[c] |
| | Salt + carnosine | ND | 2.06±0.13[c] | 3.34±0.19[d] | 8.33±0.38[d] |
| | Effect of salt | NS | NS | * | ** |
| | Effect of carnosine | * | * | ** | ** |

1 See Table 1.
Values are means ± SEM of 4 analyses.
1 Sum of individual COPs detected
ND = Not detected.
a-d Within basal and supplemental treatments, means in the same column bearing different superscripts are significantly different (ANOVA, LSD): *P<0.05, **P<0.01, NS = not significant.