

## Influence of feed enriched with natural antioxidants on the oxidative stability of broiler meat

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### Abstract

The effect of feed supplementation with natural  $\alpha$ -tocopherol, rosemary, green tea, grape seed and tomato extracts on the oxidative stability of broiler meat was investigated by measuring lipid oxidation, protein oxidation and  $\alpha$ -tocopherol content of breast muscle. Supplementation with  $\alpha$ -tocopheryl acetate at a dietary concentration of 200 ppm was used as a control. The broilers were fed a diet containing 4% linseed oil for 6 weeks. The antioxidant extracts were supplemented in single and in combinations at total concentrations of 100 and 200 ppm. For lipid oxidation, the 200 ppm  $\alpha$ -tocopheryl acetate treatment yielded the lowest TBARS values. Dose effects were observed for  $\alpha$ -tocopherol, grape seed and tomato, while no dose effect was observed for rosemary. In contrast, green tea showed a pro-oxidative effect at 200 ppm. Combinations of  $\alpha$ -tocopherol, rosemary and green tea did not reveal synergistic effects between the different extracts and strengthened the hypothesis of the pro-oxidative effect of green tea at higher doses. No differences between the different antioxidant treatments were detected for protein oxidation. The muscle  $\alpha$ -tocopherol content linearly responded to the feed  $\alpha$ -tocopherol content. No differences in  $\alpha$ -tocopherol deposition was observed between the natural or the synthetic form.

### Introduction

Oxidative damage occurs in the living animal due to an imbalance between the production of reactive oxygen or nitrogen species and the animal's defence mechanism. This defence mechanism exists of three levels. The first level consists of endogenous antioxidative enzymes and metal binding proteins, responsible for preventing the formation of free radicals, removing precursors of free radicals and deactivating catalysts. The second level, existing of endogenous and exogenous antioxidants, prevents and reduces the propagation reactions. The third consists of enzymatic systems responsible for removing and eliminating damaged molecules (Chaudière and Ferrari-Iliou, 1999; Surai, 2002). Oxidation is inherent to metabolism, but an excessive formation of reactive species in oxidation processes can cause damage to vital components in biological systems (Halliwell *et al.*, 1995). In this regard, oxidation increases due to a high intake of oxidized lipids or oxidative sensitive polyunsaturated fatty acids (PUFA) or pro-oxidants, or a low intake of nutrients involved in the antioxidant defence system (Morrissey *et al.*, 1998). Oxidation is a very general process which affects lipids, pigments, proteins, DNA, carbohydrates and vitamins (Kanner, 1994). In muscle and fat tissue oxidation continues post mortem and determines the shelf-life of meat and meat products.

It is generally accepted that lipid oxidation is one of the primary mechanisms of quality deterioration in foods and especially in meat products (Kanner, 1994; Morrissey *et al.*, 1998). The latter becomes more important because of a trend towards increasing the (long-chain) PUFA content in meat. Although proteins are the major compounds of most biological systems, little research has been performed on protein oxidation. Various reasons can be readily discerned for the dominance of lipid and DNA oxidation studies over those on protein oxidation, including the complexity of proteins as targets (20 different side-chains plus the backbone, as potential targets compared to the more limited number of reactive sites in DNA and lipids), the complexity of the products that are formed and the complexity of the mechanisms that can occur (Davies, 2005).

To maximise the oxidative stability of meat, antioxidants, mostly  $\alpha$ -tocopheryl acetate ( $\alpha$ TAc), are added to PUFA supplemented feeds. The beneficial effect of dietary  $\alpha$ TAc supplementation for the subsequent enhanced stability of lipids in foods from animal origin has been extensively reported for poultry, beef cattle, veal calves and pigs (Gray *et al.*, 1996; Jensen *et al.*, 1998). In addition, an increasing number of studies have been reported on the antioxidative properties of plant products and compounds (Halvorsen *et al.*, 2002; Pellegrini *et al.*, 2003; Schwarz, *et al.*, 2001). Several studies have already demonstrated that plant antioxidative compounds supplemented to meat post mortem are able to improve the lipid stability of the meat (Govaris *et al.*, 2003; Lau *et al.*, 2003; Nissen *et al.*, 2000). However, only a limited number of studies has been performed investigating the effect of dietary administration of natural antioxidants, other than natural  $\alpha$ -tocopherol ( $\alpha$ TC), on the oxidative stability of meat or meat products (Botsoglou *et al.*, 2002, 2003; Tang *et al.*, 2000). The aim of this study was to investigate the effect of supplementation of the broilers' diet with several extracts, rich in natural antioxidants, on the oxidative stability of meat. The effects of individual extracts were tested, as well as combinations of extracts to investigate possible additive or synergistic effects between antioxidants.

## Material and methods

### ANIMALS AND EXPERIMENTAL DESIGN

Two thousand and forty one-day old chickens (Ross 308, cocks) were divided over 60 pens and were fed for 6 weeks a diet containing 4% refined linseed oil and one of 20 antioxidants or antioxidant mixtures. Within each phase (3 phases), diets were formulated to an equal protein and energy content. In the premix no synthetic antioxidants were added, but a basal amount of 20 ppm  $\alpha$ TAc was present to meet the physiological requirements. The experimental antioxidants were mixed in the refined linseed oil prior to the feed manufacturing. Five extracts (natural  $\alpha$ TC, rosemary, green tea, grape seed and tomato; Table 1) were supplemented separately in one of two doses (100 ppm or 200 ppm), making 10 treatments. In addition,  $\alpha$ TC, rosemary and green tea were supplemented in equal proportions in all combinations two by two and all three combined at a final concentration of 100 or 200 ppm, making a further 8 treatments. Two control treatments with a mixture of synthetic antioxidants (BHT, BHA, ethoxyquin) and with or without 200 ppm  $\alpha$ TAc were also included. All antioxidant extracts were supplemented at 100 or 200 mg total product/kg feed and not as 100 or 200 mg active compounds/kg feed. These 20 antioxidant treatments were replicated in three pens (Table 3).

**Table 1 Specifications of the antioxidant extracts.**

Extract	Specification	Supplier
Synth antioxidant	BHT/ethoxyquin/BHA	NUTRI-AD International, Belgium
$\alpha$ -tocopheryl acetate	500 IU/g	NUTRI-AD International, Belgium
$\alpha$ -tocopherol	700 mg/g mixed tocopherols with 80-150 mg $\alpha$ -tocopherol, 10-30 mg $\beta$ -tocopherol, 350-450 mg $\gamma$ -tocopherol, 140-200 mg $\delta$ -tocopherol	NUTRI-AD International, Belgium
Rosemary	3% carnosic acid, 0.1% carnosol, 0.03% Me-carnosate	NUTRI-AD International, Belgium
Green tea	Cafein 4% to 8%, EGCG > 14.5%, epicatechins > 2.4%, catechins (as epicatechins) > 60%, catechins (as EGCG) 23.25% to 26.75%	NUTRI-AD International, Belgium
Grape seed	89% polyphenols, containing 3.1% catechins; 11.2% oligomeric procyanidins	NUTRI-AD International, Belgium
Tomato	No information available	NUTRI-AD International, Belgium

### SAMPLING

At the moment of slaughter, 5 birds per pen were selected with a live weight close to the average pen weight. After slaughter, the right part of the breast muscle of the 5 selected animals was pooled and minced. From this sample, sub-samples were taken for oxidative stability measurements, while the remaining was vacuum-packed and stored at  $-18^{\circ}\text{C}$ . For oxidative stability measurements, three fresh patties (approximately 100g) were overwrapped in an oxygen permeable polyethylene film and were placed in an illuminated chill cabinet (illuminance of 1000 lux, temperature  $3^{\circ}\text{C}$ ) for 10 days. Samples

were analysed for lipid and protein oxidation at day 3, 7 and 10 of display. On the frozen vacuum-packed sample, the  $\alpha$ -tocopherol content and the fatty acid profile of the meat were determined.

## ANALYSES

### Fatty acid analysis

Lipids were extracted using chloroform/methanol (2/1; v/v), modified after Folch *et al.* (1957). After methylation, fatty acid analysis was performed by gas chromatography as described by Raes *et al.* (2001). Results were expressed as g/100g fatty acid methyl esters (FAME).

### Lipid oxidation

Lipid oxidation was assessed by the TBARS-method (thiobarbituric acid reactive substances) based on Tarladgis *et al.* (1960) and is expressed as  $\mu$ g malondialdehyde/g tissue. The patties were assayed for lipid oxidation after 3, 7 and 10 days of storage in the chill cabinet.

### Protein oxidation

Oxidative damage to proteins was determined by measuring the decrease in the amount of thiol groups. Thiol groups are expressed as nmol free SH-groups/mg protein (Batifoulier *et al.*, 2002). The analyses were performed on the same samples as used for lipid oxidation.

### Muscle $\alpha$ -tocopherol content

Muscle  $\alpha$ TC levels were determined on the breast muscle sample by high performance liquid chromatography, as described by Desai (1984). Results are expressed as  $\mu$ g  $\alpha$ TC/g muscle.

## STATISTICAL ANALYSIS

Data of lipid and protein oxidation were analysed by a linear model including the fixed effects of antioxidant treatment and days of storage. The interaction term was not significant and was therefore not included in the model. TBARS values were log transformed to account for heterogeneity of variances. To compare single antioxidant treatments and to test for dose effects and possible synergistic action, specific contrasts were defined (e.g. c1 vs. c2; for number of treatments see Table 3). The analyses were performed in S-Plus 6.1.

## Results

### MEAT FATTY ACID COMPOSITION

The mean total fatty acid content of the chicken muscle was 0.94 (SD 0.19) g/100g muscle. By the use of 4% linseed oil in the diet, the PUFA content and particularly the n-3 PUFA proportion was relatively high (Table 2). No effect of the antioxidants supplemented in the diet was observed on the fatty acid profile of the breast muscle ( $p > 0.05$ ).

**Table 2 Overall mean fatty acid profile of the breast muscle.**

Fatty acid	g/100g FAME	SEM	Fatty acid	g/100g FAME	SEM
C12:0	0.03	0.001	C20:3n-6	0.52	0.01
C14:0	0.41	0.01	C20:4n-6	2.47	0.05
C16:0	17.7	0.08	C22:4n-6	0.36	0.02
C18:0	7.97	0.05	C18:3n-3	10.9	0.10
C18:1	27.3	0.12	C20:5n-3	1.17	0.02
C18:2n-6	18.7	0.08	C22:5n-3	2.65	0.05
C20:2n-6	0.43	0.01	C22:6n-3	1.43	0.04
<b>SFA</b>	<b>26.1</b>		<b>n-6</b>	<b>22.5</b>	
<b>MUFA</b>	<b>27.3</b>		<b>n-3</b>	<b>16.2</b>	
<b>PUFA</b>	<b>38.7</b>		<b>n-6/n-3</b>	<b>1.39</b>	
			<b>P/S</b>	<b>2.53</b>	

#### LIPID OXIDATION

TBARS values were significantly higher for day 7 and day 10 compared to day 3 ( $p < 0.05$ ), but day 7 and 10 were not significantly different. However, even after 10 days of chilled storage the meat samples had low TBARS values ( $< 0.8 \mu\text{g}$  malondialdehyde/g muscle; Table 3). Compared to all other antioxidant treatments, the 200 ppm  $\alpha$ -TAc treatment resulted in the lowest TBARS values ( $p < 0.05$ ), which almost remained unchanged during the 10 days storage period. For the other antioxidant treatments, some dose effects were observed. Natural  $\alpha$ TC, grape seed and tomato showed lower TBARS values at 200 vs. 100 ppm (c3 - c8; c5 - c10; c7 - c12;  $p < 0.05$ ), while rosemary showed no difference between the two doses (c4 - c9;  $p > 0.05$ ). In contrast, TBARS-values were higher for the 200 ppm compared to the 100 ppm green tea treatment (c6 - c11;  $p < 0.05$ ). The combination of rosemary and green tea inhibited lipid oxidation more when supplemented in a combined dose of 200 ppm compared to 100 ppm (c15 - c18;  $p < 0.05$ ). No dose effect was observed when  $\alpha$ TC was supplemented in a combined dose with rosemary or green tea (c13 - c16; c14 - c17,  $p > 0.05$ ). The combination of  $\alpha$ TC, rosemary and green tea at a combined dose of 200 ppm yielded significantly lower TBARS values compared to the combined dose of 100 ppm (c19 - c20;  $p < 0.05$ ).

**Table 3 Mean values for lipid oxidation ( $\mu\text{g}$  malondialdehyde/g meat) and protein oxidation (nmol free SH groups/mg protein) of the breast muscle stored in a chill cabinet during 3, 7 or 10 days.**

Antioxidant treatment	Lipid oxidation				Protein oxidation		
	Day 3	Day 7	Day 10	SEM	Day 3	Day 10	SEM
1 synthetic	0.14	0.23	0.28	0.03	64.6	89.3	9.49
2 synthetic + 200 ppm $\alpha$ TAc	0.07	0.14	0.09	0.02	74.5	72.2	1.45
3 100 ppm $\alpha$ -tocopherol ( $\alpha$ TC)	0.15	0.24	0.34	0.04	71.4	69.7	2.66
8 200 ppm $\alpha$ -tocopherol	0.10	0.20	0.18	0.02	73.2	72.0	0.70
4 100 ppm rosemary (ros)	0.16	0.34	0.32	0.03	74.2	71.5	2.09
9 200 ppm rosemary	0.17	0.30	0.32	0.04	76.4	70.9	1.86
5 100 ppm grape seed	0.28	0.51	0.59	0.08	71.3	73.7	1.20
10 200 ppm grape seed	0.18	0.36	0.34	0.04	77.5	69.4	2.66
6 100 ppm green tea	0.15	0.24	0.28	0.03	72.8	74.7	2.65
11 200 ppm green tea	0.52	0.77	0.72	0.13	73.1	69.6	2.09
7 100 ppm tomato	0.23	0.46	0.49	0.06	69.2	74.1	1.38
12 200 ppm tomato	0.19	0.29	0.28	0.03	75.7	70.7	2.67
13 $\alpha$ TC + ros (100 ppm)	0.15	0.31	0.26	0.03	72.8	71.9	3.28
16 $\alpha$ TC + ros (200 ppm)	0.18	0.18	0.32	0.06	74.5	76.2	1.01
14 $\alpha$ TC + tea (100 ppm)	0.14	0.22	0.21	0.02	72.1	66.0	2.42
17 $\alpha$ TC + tea (200 ppm)	0.16	0.28	0.31	0.03	75.2	72.0	2.85
15 ros + tea (100 ppm)	0.34	0.61	0.65	0.14	63.8	71.8	2.61
18 ros + tea (200 ppm)	0.20	0.20	0.25	0.03	76.4	72.1	2.84
19 $\alpha$ TC + ros + tea (100 ppm)	0.18	0.33	0.30	0.03	77.1	73.5	2.77
20 $\alpha$ TC + ros + tea (200 ppm)	0.11	0.18	0.23	0.02	73.9	66.8	3.41

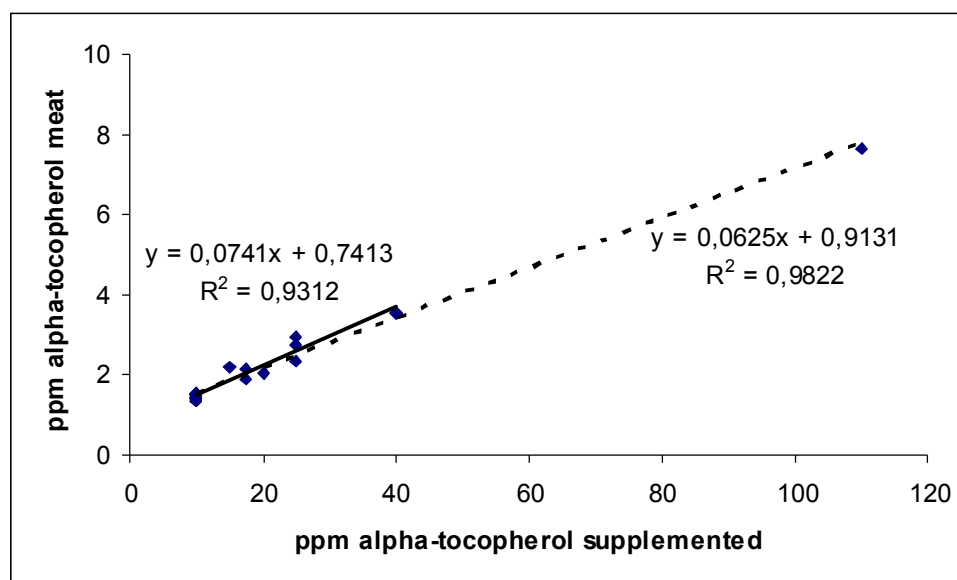
#### PROTEIN OXIDATION

The results for protein oxidation, measured by the amount of thiol groups disappearing during storage, are presented in Table 3. If protein oxidation occurs, thiol groups disappear. However, in this study no differences in thiol content were observed in the patties displayed for 3 or for 10 days ( $p > 0.05$ ) and no differences between antioxidant treatments were apparent.

#### MEAT A-TOCOPHEROL CONTENT

Natural  $\alpha$ TC was supplemented at different doses as indicated in Table 3. Furthermore, all diets contained 20 ppm  $\alpha$ TAc (50% active compounds) and one control treatment delivered 200 ppm  $\alpha$ TAc (50% active compounds) extra. Figure 1 shows a good correlation between the dietary natural  $\alpha$ TC content (ppm active compounds) and the amount of  $\alpha$ TC deposited in the muscle tissue ( $r^2 = 0.93$ ).

Only about 6 to 7.5 % of the supplemented  $\alpha$ TC (as natural  $\alpha$ TC or as synthetic  $\alpha$ TAc) was incorporated in the meat. The supplementation of  $\alpha$ -tocopherol, delivered as natural or as acetate form gave no differences in incorporation. The other antioxidants, combined with  $\alpha$ TC, did not influence the  $\alpha$ TC deposition in muscle tissue.



**Figure 1 Relationship between the feed ( $\alpha$ TC+  $\alpha$ TAc) content (active compounds) and the  $\alpha$ TC content in breast muscle.**

## Discussion

### FATTY ACID COMPOSITION

Compared to other meats, chicken meat is relatively abundant in PUFA, including the key n-3 fatty acids, since diets of fast growing broilers are generally rich in PUFA (Asghar *et al.*, 1990; Rhee *et al.*, 1996). Furthermore, the fatty acid composition of the diet is reflected in the fatty acid composition of the meat. In this study, the linolenic acid content was high as a result of feeding 4% linseed oil, and in line with other studies that reported an increase in the degree of unsaturation after feeding with different kinds of oils (Cortinas *et al.*, 2004; Jahan *et al.*, 2004; Kahraman *et al.*, 2004; Lauridsen *et al.*, 1997; Ozpinar *et al.*, 2002). An unsaturated feed fat source was chosen to boost oxidation in this study. The fatty acid composition was not influenced by the different antioxidant treatments.

### LIPID OXIDATION

An increase in PUFA content influences lipid oxidation and can affect colour, flavour and subsequently oxidative stability during suboptimal storage (Basmacioglu *et al.*, 2004). However, lipid oxidation can be prevented by the use of dietary antioxidants. It has been clearly demonstrated that  $\alpha$ TAc supplementation resulted in good oxidative stability (e.g. Botsoglou *et al.*, 2003; Coetzee and Hoffman, 2001; Lopez-Bote *et al.*, 1998). However, the last years the possibilities of dietary supplementation of other natural antioxidant extracts are increasingly investigated.

Our results showed an antioxidative dose effect for natural  $\alpha$ TC, tomato and grape seed. These findings confirm the work of Lau *et al.* (2003) for grape seed and of Coetzee and Hoffman (2001) for  $\alpha$ TC. For green tea, a pro-oxidative dose effect was observed, which is in contrast with the results of Tang *et al.* (2000), who observed a clear antioxidative dose-response effect. However, the different effects between our study and the one of Tang *et al.* (2000) could be due to a different content of the catechins present in the green tea extracts. Also other plant extracts, e.g. rosemary and sage extracts at 500 ppm (Lopez-Bote *et al.*, 1998), oregano and rosemary essential oils at 150 and 300 ppm (Basmacioglu *et al.*, 2004) or at 100 and 200 ppm (Botsoglou *et al.*, 2003; Papageorgiou *et al.*, 2003), and a mixture of marigold, purple coneflower, black currant and yellow bark at 1000 ppm (Botsoglou *et al.*, 2004) have been shown to improve the oxidative stability of chicken meat.

One of the objectives of our experiment was to investigate possible synergistic effects by making combinations vs. single antioxidant additions. The combination of  $\alpha$ TC and rosemary at both 100 and 200 ppm did not yield lower TBARS values compared to the single  $\alpha$ TC and rosemary addition ( $p>0.05$ ), which may suggest no synergistic action at these doses. The combination of  $\alpha$ TC and green tea at 200 ppm showed no lower TBARS values compared to the single additions at 100 ppm ( $p>0.05$ ). However, this combination yielded significantly higher TBARS values compared to 200 ppm  $\alpha$ TC alone and significantly lower TBARS values compared to 200 ppm green tea alone. This suggests that green tea delivered no extra antioxidant action when it was combined with  $\alpha$ TC at these doses. The combination of green tea and rosemary at 200 ppm did not result in significantly lower TBARS values compared to these single antioxidants at 100 ppm ( $p>0.05$ ). However, this combination showed significantly lower TBARS values compared to 200 ppm green tea alone, which strengthens the pro-oxidative effect of green tea. Few data are available about possible synergistic effects of natural extracts on the oxidative stability of meat. Basmacioglu *et al.* (2004) reported a synergistic effect for oregano and rosemary essential oils in broilers at a dose of 300 ppm. Also a synergistic action between oregano oil and  $\alpha$ TAc (200 ppm) resulted in a higher oxidative stability than 200 ppm  $\alpha$ TAc alone in turkeys (Botsoglou *et al.*, 2003; Papageorgiou *et al.*, 2003).

Literature data are also available on the stabilising effects of post mortem added antioxidants. Higgins *et al.* (1998) observed that endogenous  $\alpha$ TC was much more effective than exogenously added  $\alpha$ TC, because dietary addition allows  $\alpha$ TC to be positioned closely to unsaturated lipids in membranes to exert its greatest effect as an antioxidant (Lau *et al.*, 2003). Post mortem addition of oregano oil also seemed to exert an antioxidative action. However, dietary supplementation was also more effective for this antioxidant (Govaris *et al.*, 2003). Also grape seed extracts exerted some antioxidative action post mortem (Lau *et al.*, 2003).

#### PROTEIN OXIDATION

As oxidative stress should not only be evaluated by lipid oxidation (Dotan *et al.*, 2004), protein oxidation was also investigated in this study. However, no differences were observed between 3 and 10 days of storage and between antioxidant treatments. This is in contrast with the work of Mercier *et al.* (1998) that found differences in protein oxidation after feeding with different dietary fat and different levels of  $\alpha$ -TAc in turkey meat.

#### MUSCLE A-TOCOPHEROL CONTENT

The  $\alpha$ TAc is only effective after hydrolysis in the gut and does not protect the feed from oxidation (Jensen *et al.*, 1998). From the regression curve, no preference is observed between deposition of  $\alpha$ TAc or  $\alpha$ TC. Although the refined linseed oil was not stabilised, the possible consumption of  $\alpha$ TC in the feed, to protect it from oxidation, seemed not to influence its deposition in the muscle.

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