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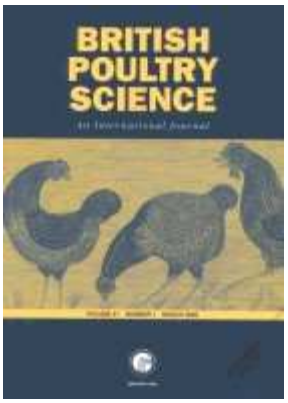

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Effects of α-tocopheryl acetate and β-carotene dietary supplementation on the antioxidant enzymes, TBARS and sensory attributes of turkey meat

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RUNNING TITLE: **OXIDATION AND SENSORY EVALUATION**

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Abstract 1. The aim of the study was to evaluate the effects of α-tocopheryl acetate (50 mg/kg) and β-carotene (15 mg/kg) dietary supplementation on the oxidative status of raw turkey breast and leg muscles assessed by thiobarbituric acid test values, the vitamin E levels and the antioxidant enzyme activities. In parallel, a quantitative descriptive sensory analysis was carried out on cooked, stored and reheated samples.

2. Vitamin E was present in sufficient quantity to reduce oxidation, since iron-induced reactive substances (TBARS) were significantly lower in antioxidant supplemented treatments. The results suggested that the presence of β-carotene in the diet limits the accumulation of α-tocopherol in turkey muscles.

3. In the present study, there was no conclusive relationship between dietary antioxidant supplementation and endogenous antioxidant enzyme activities.

4. Sensory evaluation showed that a longer supplementation time and dose may be necessary in turkeys to prevent meat from rancidity and warmed-over flavour (WOF). Leg pastiness and stringiness were modified by dietary antioxidant supplementation, indicating the possible synergism between antioxidants and cysteine proteinases in the perception of meat quality.

5. Given the modern trends that lead consumers to increase their consumption of poultry meat, it would be interesting to evaluate the commercial potential and cost effectiveness of routine dietary antioxidant supplementation.

INTRODUCTION

World consumption of turkey meat has increased not only because of its sensory attributes of taste and texture but its moderate levels of total lipids, saturated fatty acids and cholesterol. These characteristics are considered to provide beneficial effects on human health, especially in the prevention of cardiovascular disease

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(Krauss *et al.*, 2001); however, on the other hand, they increase the susceptibility of meat to lipid oxidation (Morrissey *et al.*, 1998). Feeding antioxidant supplemented diets and controlling conditions of rearing and slaughtering the antioxidative status in the living animal may be influenced, producing beneficial results for both the consumer and the processing industry. Dietary supplementation with vitamin E has been widely reported to be effective in protecting poultry muscle against oxidative damage (Carreras *et al*., 2004; Grau *et al.,* 2001; Maraschiello *et al.,* 1999; Nam *et al*., 2003). Results on the effectiveness of β-carotene as antioxidant are not conclusive, since its effect depends on the dose and on the type of fat fed (King *et al.,* 1995; Ruiz *et al.,*1999).

Lipid oxidation can give rise to rancidity and the formation of undesirable odours and flavours, which affect the sensory and nutritional values of products. The term warmed-over flavour (WOF) (Tims and Watts, 1958) is used to describe the sensory perception associated with the rapid development of oxidised flavours in precooked, stored and reheated meat. WOF becomes apparent within a short period of time, whereas common rancidity develops during lengthy storage. The use of a trained panel has been demonstrated (Guerrero, 2000) to be appropriate for assessing quality attributes, as they may be perceived by the consumers, and to complement results obtained by analytical methods. In this sense, the oxidative stability of meat can be studied assessing the enzymatic activities of glutathione peroxidase (GSHPx), catalase (CAT) and superoxide dismutase (SOD) that constitute an antioxidant cellular enzymatic system. Although, the thiobarbituric acid (TBA) test has been criticised for its lack of sensibility and specificity

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(Botsoglou *et al*., 1994), it seems to be an appropriate method for routine assessment of secondary oxidation involved in rancidity development.

The aim of the current work was to evaluate the effect of diet modifications with antioxidant supplementation on the oxidative status of raw turkey breast and leg muscles assessed by the thiobarbituric acid test and antioxidant enzyme activities. In parallel, sensory analyses were carried out on cooked samples. Sensory analyses done together with chemical measurements are one of the most appropriate tools to assess the lipid oxidation status of meat and meat products. Our goal was to complement previous results obtained on the oxidative stability of broiler muscles (Carreras *et al*., 2004).

MATERIAL AND METHODS

Birds and dietary treatments

A total of 30 turkeys of BUT 9 strain were selected. They were placed in flat-deck batteries (6 m^2). The animals were raised according to routine practices in terms of light and temperature. They were all fed on a basal diet containing lard as saturated fat (Table 1) throughout the experiment, which lasted 12 weeks. Animals were distributed randomly in three experimental treatments: T1 (control group) that was only given the basal diets; T2 turkeys were given the basal diets supplemented with 50 mg/kg of α-tocopheryl acetate during the last 4 weeks before slaughter; T3 birds were fed on the basal diets supplemented with 50 mg/kg of α-tocopheryl acetate and 15 mg/kg of β-carotene for the last 4 weeks before slaughter. The doses of α-tocopheryl acetate and β-carotene were chosen to take into account the expected protective effect that dietary supplementation of two antioxidants may produce in turkey meat. Previous studies pointed out the antioxidant effect of dietary supplementation with 15 mg/kg of β-carotene against

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the prooxidant results found with 50mg/kg of β-carotene supplementation in poultry meat (Maraschiello, 1998., Ruiz *et al*., 1999).

Table 1 near here

Ten animals from each treatment were slaughtered at a commercial processing plant. The breast and leg were removed, vacuum packed in aluminium foil bags (MET/MET Sacoliva, Spain) and stored at –20 ºC until analysis. The left breast and leg of each bird was used for the chemical analyses and the other half of each animal was used for the sensory analyses.

Preparation of antioxidant enzymatic extract

The enzymatic extract was prepared according to the procedure of DeVore and Greene (1982). A portion of tissue was homogenised with 50 mM Tris-HCl buffer, pH 7.0 (1/5 w/v). The homogenate was centrifuged at 17000 *g* for 30 min at 4 ºC. The supernatant was recovered and filtered through deactivated glass wool. The subsequent centrifugation of the filtrate, carried out at 100000 *g* for 1 h at 4 ºC, gave the final supernatant extract used for SOD, CAT and GSHPx activity assays. The protein content of the extract was determined by the procedure of Lowry *et al*. (1951).All the assays were done in duplicate.

Measurement of GSHPx activity

The GSHPx assay (DeVore and Greene, 1982) medium consisted of 40 mM potassium phosphate, pH 7.0, 0.5 mM EDTA, 1 mM NaN_3 (sodium azide), 0.5 units of glutathione reductase, 1 mM reduced glutathione, 0.15 mM NADPH, 0.12 mM H_2O_2 and 150 µl of the tissue extract, with a total volume of 1.5 ml. The mixture was incubated in a water bath at 30 ºC for 5 min before the addition of hydrogen peroxide. The decrease of absorbance at 340 nm was recorded for over 5 min. The activity was expressed as nmol of NADPH oxidised per minute per mg of protein.

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Measurement of SOD activity

Superoxide dismutase (SOD) activity was measured according to the method of Marklund and Marklund (1974), based on the ability of SOD to inhibit the autoxidation of pyrogallol. Fifty µl of tissue extract were incubated at 30 ºC for 10 min with 0.2 mM pyrogallol and 50 mM tris-cacodylic acid buffer, pH 8.2, containing 1 mM diethylenetriaminepentaacetic acid (DTPA) with a total volume of 2 ml. The rate of autoxidation was determined by the absorbance measured at 420 nm. The enzyme activity was calculated according to a SOD standard curve (0 to 200 ng). One unit of enzyme activity was defined as the amount of the enzyme that inhibited the autoxidation of pyrogallol by 50 %. Activity was expressed as units of SOD per mg of protein

Assay of CAT activity

Catalase (CAT) activity was determined by measuring the decrease in hydrogen peroxide at 240 nm for 5 min (Mei *et al*., 1994). The assay medium consisted of 11 mM H_2O_2, 50 mM potassium phosphate buffer, pH 7.0, and 100 µl of tissue extract with a total volume of 3 ml. The blank mixture contained potassium phosphate buffer, pH 7.0, instead of extract. A unit of CAT activity was defined as the amount of CAT needed to decompose 1 µmol H_2O_2 per min. Activity was expressed as µmol of H_2O_2 decomposed per minute per mg of protein.

Measurement of lipid oxidation by the iron-induced TBA test

The iron-induced TBA test carried out to measure lipid oxidation in raw muscles was a modification of the method of Kornbrust and Mavis (1980). Each sample (3 g) was homogenised in 27 ml of 1.15 % KCl. A 200µl aliquot of the homogenate was mixed with 1 ml of 80 mM Tris/maleate buffer, pH 7.4, 400 µl of ascorbic

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acid and 400 µl of ferrous sulphate and incubated for different periods of time (0, 50, 100 and 150 min) in a 37 ºC water bath with gentle stirring. Then, 4 ml of 26 mM TBA, 0.92 M TCA and 0.8 mM HCl were added and held for 15 min in boiling water. Different incubation times were tested in order to determine which showed higher correlation coefficients with sensory rancidity and discriminated better between treatments. The samples were cooled and the absorbance was recorded spectrophotometrically at 532 nm. Blanks containing all the reactants except the homogenate were also measured. The extent of lipid oxidation was expressed as nmol of malonaldehyde (MDA) per mg of tissue. TBARS were calculated after 150 min of incubation.

Determination of α-tocopherol levels

A portion of muscle was sonicated in n-hexane/2-propanol (3:2, v/v) to achieve α-tocopherol extraction. The sample was centrifuged and the supernatant was evaporated to dryness in a stream of nitrogen. The residue was redissolved in 1ml of n-hexane/ethyl acetate (80:20, v/v). A 20 µl aliquot of the filtered extract was injected into an HPLC system. Samples and standards (5 and 10 ng/µl of α-tocopheryl acetate in mobile phase) were analysed by normal phase HPLC using a LKB Bromma System (Stockholm, Sweden) with an aminopropylsilica NH_2-NP (5 µm, 250 mm x 4.6 mm i.d.) column (Supelco-Sigma, USA). The mobile phase consisted of n-hexane/ethyl acetate (80:20, v/v) at a flow rate of 1.2 ml/min. Detection was carried out by measurement of the fluorescence (290-330 nm) (Casademont and García Regueiro, 2000).

Sensory analysis

Ten breasts and legs per treatment were evaluated. The samples were vacuum-packed and cooked for 80 min and 120 min respectively, in an 80 ºC water bath.

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The exudate was removed and each breast was cut into 6 pieces, one for each assessor. *M. gastrocnemius lateralis* and *M. gastrocnemius medialis* of each leg were dissected and used for flavour and texture evaluation, respectively. In this case, one complete leg of each treatment per assessor was evaluated in each session. The samples were randomly coded and stored for 1 d at 4 ºC in order to facilitate rancidity development. Before sensory analysis, samples were reheated for 25 min at 65 ºC in an electric oven and served immediately in isothermic containers at 70 ºC to 6 previously trained sensory panellists according to Guerrero *et al.* (2000).

Two flavour (rancidity and WOF) and 5 texture (initial juiciness, hardness, pastiness, stringiness and tooth adhesion) descriptors were evaluated. Rancid flavour was described as the intensity of flavour perception similar to that of an old olive oil odour; warmed-over flavour as the oxidised flavour of reheated meat; initial juiciness as the moisture released by the product in the mouth as a result of initial chewing; hardness as the force required to bite through the sample; pastiness as the pasty feeling inside the mouth; stringiness as the perception of long and parallel coarse particles in the meat during chewing and tooth adhesion as the perception of mouth residue that remains stuck to the teeth once chewing is finished.

Attributes were quantified on a rating scale from 0 to 10 (where zero is absence and 10 maximum intensity). Sensory evaluations were conducted in a standardised sensory testing room (ISO, 1988) equipped with red lights to mask colour differences between samples. A complete block design was used. The order of presentation of samples and the first-order carry-over effects were

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blocked (Macfie *et al.*, 1989), as well as the piece served to each assessor in each of the tasting sessions carried out (Guerrero, 2000).

Statistical analysis

All the data were analysed using the MEANS, GLM and CORR procedures of the SAS statistical package (SAS Institute, 2000). An analysis of variance (ANOVA) was carried out for chemical data on each tissue (breast and leg). The ANOVA (GLM) model included the treatment as fixed effect. An additional ANOVA was carried out to check the existence of significant ($P < 0.05$) differences between the different tissues. In this case, for each treatment, the model included the tissue as a fixed effect.

For sensory data the ANOVA was performed over the mean values (6 assessors) for each piece of breast. In this case the model included the treatment, the taste session and their interaction as fixed effects. For legs, the model included the treatment, the assessor and the taste session nested to the assessor as a fixed effects. No significant interaction was observed between treatment and assessor. In all cases, mean separation was performed using the Least Significant Difference (LSD) test.

Correlation analysis between all the available information was carried out within each treatment.

RESULTS AND DISCUSSION

Antioxidant enzyme activities

Table 2 shows the results of GSHPx, CAT and SOD activities in raw breast and leg samples. The activity of antioxidant enzymes in meat is affected by such factors as species, genotype, phenotype and the muscle type (Daun and Akesson, 2004; Sárraga *et al.,* 2002; Hernández *et al*., 2004). In our study, leg muscles

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exhibited more CAT and GSHPx activities than breast samples, while the differences in SOD activity were not significant. Some reports (Lee *et al*., 1996; Renerre *et al.,*1999) described higher antioxidant enzyme activities in oxidative (leg) than in glycolytic muscles (breast) from turkey. This could be considered as a protective system preventing or delaying the onset of oxidative stress in susceptible muscles. Moreover, according to Daun and Akesson (2004), the higher GSHPx activity observed in legs may be due to the greater content of total and soluble selenium found in oxidative turkey muscles. Table 2 near here

GSHPx and SOD activities were not influenced by antioxidant supplementation. There are several contradictory reports in the literature about the capability of endogenous antioxidant enzymes to induce a compensatory activity in response to the exposure of tissue to an oxidative stress environment. Some authors reported an inverse relationship between dietary vitamin E content and GSHPx activity (Yamauchi *et al*., 1984; Maraschiello *et al.,* 1999) contrary to the findings of Renerre *et al.* (1999) in muscles from turkeys receiving 30 and 200 mg/kg of vitamin E and Rojas *et al*. (1996) in guinea pig heart where no effect on antioxidant enzyme activities was reported. The observed relationship between vitamin E and the enzyme activities may be related to parameters such as dietary selenium or the level of unsaturation of the fat included in the diets.

Differences among treatments were found only in CAT activity of breast muscles, increasing significantly when antioxidants were supplemented together (T3). A positive correlation between CAT and SOD activities (r = 0.66) was also observed. In the same way, GSHPx was correlated with SOD (r = 0.74) in leg samples from T 3, indicating a possible synergism between the enzymes. This can

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be explained because SOD catalyses the superoxide anion. The H_2O_2 produced is removed by CAT and GSHPx activities

From our results, it is not possible to use any of these enzyme activities as indicators of the oxidative stress prevailing in the muscle. In our conditions there was no conclusive relationship between the antioxidant supplemented level and the endogenous antioxidant enzymes system activity.

Iron-induced TBARS and vitamin E levels

The vitamin E and TBARS contents of turkey breast and leg muscles obtained for each treatment are shown in Table 3. According to previous results (Ruiz, 1999; Ruiz *et al*., 1999) β-carotene was not detected in meat samples from broilers fed on diets with 15 mg/kg; consequently β-carotene content was not measured in the present study.

Table 3 near here

From our earlier results, vitamin E levels detected in turkey muscles were lower than those obtained in chicken (Carreras *et al*., 2004), in spite of the fact that supplementation with the antioxidant was higher in turkey (50 mg/kg) than in broiler (30 mg/kg) experimental treatments. In both studies, birds were fed on a diet containing lard as saturated fat, which is the usual practice in feed formulation (Table 1), in order to avoid the influence of dietary fat (Maraschiello *et al*., 1999; Grau *et al.,* 2001) and to evaluate the effect of the antioxidants. Marusich *et al.* (1975) found that vitamin E concentrations in turkey liver and breast muscles were 1/5 and 1/3 respectively, of those from broilers fed similar dietary levels. In more recent studies, some authors pointed out that turkey meat has a weak ability to store α-tocopherol in cell membrane phospholipids (Wen *et al*., 1997); in consequence, longer periods of supplementation or higher doses of antioxidant could be necessary to reach similar levels as with other kinds of

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poultry (Sklan *et al*., 1983; Morrissey *et al*., 1998). Sklan *et al*. (1983) suggested that the low levels of vitamin E could be due to a high production and excretion of tocopheryl glucoronides in turkey.

The accumulation of vitamin E in legs was more than twice that in breasts even in the control group that received only 30 mg/kg of vitamin E from the basal diet (Table 1). Some authors (Jensen *et al*., 1998; Gatellier *et al.,* 2000) have reported that leg muscles are prone to oxidative processes and, therefore, showed higher activities of antioxidant enzyme system (Table 2) and displayed higher general antioxidant status than breast in agreement with the results obtained with both vitamin E and TBARS levels (Table 3). It is worth noting that TBARS values are higher in leg than in breast muscles and antioxidant supplemented treatments (T2 and T3) showed significantly lower TBARS levels than the control group in both types of muscles (Table 3).

Vitamin E content in muscles from turkeys supplemented only with α-tocopheryl acetate (T2) increased ($P < 0.05$) compared with control samples (T1), whereas no significant differences in vitamin E levels were found between samples from T3 (birds fed on diets supplemented with 50 mg of α-tocopheryl acetate/kg plus 15 mg of β-carotene/kg) and T1. These results suggest that the presence of β-carotene in the diet seems to limit the accumulation of α-tocopherol in turkey muscles.

Sensory evaluation

The effects of antioxidant supplemented diets on sensory parameters (2 flavour parameters and 5 texture attributes) of cooked, 24 h stored and reheated turkey samples are shown in Table 4. WOF and rancidity attributes were not significantly affected by experimental treatments. Samples from treatments 2 and

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3 showed a tendency to lower WOF values than samples from control group. The same trend was observed in rancidity scores from leg muscles. This tendency was not detected in breast samples, possibly because of the action of the β-carotene supplementation. These results may indicate that vitamin E was present in the correct quantity to reduce oxidation, since TBARS values were significantly lower, but not at a sufficient level to protect meat from rancidity and WOF detectable by trained panellists.

Table 4 near here

Few significant differences were observed in the texture attributes assessed. Breast samples did not presented any statistical differences between treatments. A tendency to a high degree of hardness and stringiness, and less juiciness was observed in breasts from T2. Significant differences were found in pastiness and stringiness evaluations of leg muscles. The stringiness was lower in leg muscles of turkeys fed with antioxidant supplemented diets compared to control samples ($P < 0.05$), whereas T3 presented significantly higher pastiness than control group. An early study (Sárraga and García Regueiro, 1999) about the relationship between membrane lipid oxidation and cysteine proteinases in broiler breasts suggested that cathepsins B and L activities were favoured by vitamin E supplementation. These enzyme activities are closely related to texture of meat and meat products (Arnau *et al.*, 1998; Gil *et al.,* 1999). In this sense, higher proteolytic activity could have produced greater pastiness as a result of the protein breakdown and lower stringiness caused by the myofibrillar structure breakdown.

In summary, a dose and a longer supplementation time might be necessary for turkeys to achieve equivalent perception of quality and protection against oxidative stress as in our previous studies with broilers (Carreras *et al.*, 2004). Given the effectiveness of vitamin E dietary supplementation for poultry and the

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resultant improvement in overall meat quality, along with the modern trends that lead consumers to avoid food rich in saturated fatty acids, it would be interesting to evaluate the commercial potential and cost effectiveness of these nutritional methods.

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Table 1. *Composition and stimated nutrient content of the basal diets*

Ingredient	0 to 4 weeks (g/kg)	5 to 8 weeks (g/kg)	9 to 12 weeks (g/kg)
Wheat	418.34	484.63	564.77
Lard	34.09	39.78	60.00
Soybean meal 48%	473.63	416.18	328.16
Extruded soybean	20.0	20.0	-
DL-methionine	2.27	1.69	1.16
L-lysine	0.37	0.95	1.90
Calcium carbonate	19.50	11.09	22.63
Dicalcium phosphate	23.64	18.46	15.01
Sodium chloride	3.74	3.18	2.38
Choline chloride 50%	0.42	0.04	-
Monensin (mg/kg)	90	90	-
Minerals and vitamins[1]	4.00	4.00	4.00
ME (MJ/kg)	11.7	12.1	12.55
Crude protein (g/kg)	280	260	220
Crude fibre (g/kg)	31	31	28

[1]One kg of feed contained the following: retinol, 4128 µg; cholecalciferol, 60 µg; all-rac-α-tocopheryl acetate, 30 mg; menadione, 3 mg; thiamine, 2.2 mg; riboflavin, 8 mg; pyridoxine, 5 mg; cyanocobalamin, 11 µg; folic acid, 1.5 mg; biotin, 150 µg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; ethoxyquin, 150 mg.

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Table 2. *GSHPx[1] (nmol NADPH/min per mg protein), CAT (µmol H_2O_2/min per mg of protein) and SOD (units of SOD/mg of protein) activities in tissues from turkeys fed supplemented diets[2]*

	GSHPx			CAT			SOD		
	Breast	**Leg**	**RMSE**	**Breast**	**Leg**	**RMSE**	**Breast**	**Leg**	**RMSE[3]**
T1	6.14[x]	10.70[x]	1.70	0.17[bx]	0.67[x]	0.20	3.58	4.01	0.55
T2	5.95[x]	11.50[x]	1.35	0.21[abx]	0.69[x]	0.20	3.44	4.09	0.95
T3	6.12[x]	11.40[x]	1.60	0.25[ax]	0.76[x]	0.30	3.42	4.11	0.70
RMSE[3]	1.03	1.37		0.06	0.10		0.32	0.47	

[ab]Means within the same column not sharing a common superscript are significantly different ($P < 0.05$).
[x] Means within the same row treatment and enzyme differ significantly ($P < 0.05$) between tissues.
n = 10 for each dietary treatment.
[1] GSHPx: glutathione peroxidase; CAT: catalase; SOD: superoxide dismutase.
[2] Experimental treatments: **T1**: basal diet; **T2**: basal diet supplemented with 50 mg of α-tocopheryl acetate/kg of diet; **T3**: basal diet supplemented with 50 mg of α-tocopheryl acetate plus 15 mg of β-carotene/kg of diet.
[3] RMSE: root mean square error.

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Table 3. *α-Tocopherol (μg/g of tissue) concentration and TBARS[1] (nmol of MDA/mg of tissue) values in tissues from turkeys fed on supplemented diets[2]*

	Vitamin E			TBARS		
	Breast[x]	**Leg[x]**	**RMSE**	**Breast[x]**	**Leg[x]**	**RMSE[3]**
T1	0.66[b]	1.61[b]	0.14	0.13[a]	0.51[a]	0.08
T2	0.80[a]	2.06[a]	0.27	0.08[b]	0.27[b]	0.09
T3	0.70[ab]	1.74[b]	0.14	0.09[b]	0.28[b]	0.14
RMSE[3]	0.12	0.24		0.03	0.15	

[x] All means values were statistically different ($P < 0.05$) between tissues in all treatment and for both parameters determined.
[ab]Means within the same column not sharing a common superscript are significantly different ($P < 0.05$).
n = 10 for each dietary treatment.
[1] TBARS: Thiobarbituric-reactive substances; MDA: malonaldehyde.
[2] Experimental treatments: **T1**: basal diet; **T2**: basal diet supplemented with 50 mg of α-tocopheryl acetate/kg of diet; **T3**: basal diet supplemented with 50 mg of α-tocopheryl acetate plus 15 mg of β-carotene/kg of diet.
[3] RMSE: root mean square error.

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Table 3. *Flavour and texture descriptors in cooked tissues from turkeys fed supplemented diets*[1]

	Breast				Leg			
	T1	**T2**	**T3**	**RMSE**[3]	**T1**	**T2**	**T3**	**RMSE**[3]
Rancidity	1.9	1.4	1.9	0.7	1.6	1.5	1.3	1.2
WOF[2]	2.4	2.2	2.1	0.6	3.5	3.0	2.1	1.5
Juiciness	3.4	3.2	3.4	0.6	3.2	3.5	2.8	1.1
Hardness	3.8	4.2	3.8	0.9	4.5	4.2	4.7	1.3
Pastiness	2.6	2.4	2.4	0.8	1.6^{b}	2.0^{ab}	2.7^{a}	0.9
Stringiness	3.3	3.6	3.3	0.8	4.6^{a}	3.6^{b}	3.5^{b}	1.0
Tooth adhesion	3.3	3.0	3.1	0.5	3.4	2.4	2.9	1.0

[ab]Means within the same column not sharing a common superscript are significantly different ($P < 0.05$).
Breasts: n = 10 for each dietary treatment. Legs: n = 6 for each dietary treatment.
[1] Experimental treatments: **T1**: basal diet; **T2**: basal diet supplemented with 50 mg of α-tocopheryl acetate/kg of diet; **T3**: basal diet supplemented with 50 mg of α-tocopheryl acetate plus 15 mg of β-carotene/kg of diet.
[2] WOF: warmed-over flavour.
[3] RMSE: root mean square error.

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