ANTIOXIDANT ENZYME ACTIVITIES AND TBARS OF BREAST MUSCLES FROM BROILERS FED DIFFERENT NATURAL ANTIOXIDANT SUPPLEMENTED DIETS

Carreras, I., Esteve García, E*., García Regueiro, J.A., Sárraga,C.
*Mas Bové Centre (IRTA). Dpt. of Aminal Nutrition. Crta. Reus-El Morell, Km 45. 43280 Reus. Tarragona. (Spain)
Meat Technology Centre (IRTA). Food Chemistry Unit. Granja Camps i Armet s/n. 17121 Monells. Girona (Spain)

Background

Lipid oxidative processes affect meat quality and safety. Lipid oxidation is mainly due to oxidation of membranal phospolipids. To protect themselves against the damage induced by reactive oxygen species (ROS), the cells developed protective antioxidant systems. These systems include preventive antioxidant enzymes such gluthathione peroxidase (GSHPx), catalase (CAT) and superoxide dismutase (SOD). α-Tocopherol (vitamin E) and carotenoids (β-carotene and lycopene) are into a second group of protective agents (Halliwell et al., 1995). The balance of antioxidants vs prooxidants is maintained in the living animal, but this is not the case for meat and meat products (postmortem conditions)

Dietary antioxidant supplementation is an efficient mean for increasing the oxidative stability of meat (Buckley and Morrissey, 1994). In this sense, the use of natural antioxidant has increased during last years because of the word wide trend to avoid the use of synthetic food additives (Frankel, 1993).

Several studies suggest that α-tocopherol is the most effective lipophilic antioxidant to protect muscle against oxidative damage. Results of the effectiveness of β-carotene are not so clear (Ruiz et al., 1999). In vitro studies, the tetraterpenic carotenoid lycopene have shown to be one of the most powerful antioxidant (Stahl and Sies, 1996), but any study on the use of this compound as animal nutrition dietary antioxidant has been carried out.

Objetives

GSHPx, CAT and SOD activities and TBARS (Thiobarbituric Acid Reactive Substances) levels in breast and thigh muscles from broilers has been evaluated. The consequences of feeding natural antioxidants (α-tocopheryl acetate, β-carotene and lycopene) on the enzyme activity and lipid oxidation were also investigated.

Methods

Broilers were divided into four groups and fed with different diets. One group (control) was fed only a basal diet including 20 mg/Kg of α-tocopheryl acetate and lard as fat source. A second group was fed the basal diet supplemented with 30 mg/Kg of α-tocopheryl acetate. A third group was fed the basal diet supplemented with 30 mg/Kg of α-tocopheryl acetate and 15 mg/Kg of β-carotene, and a fourth group was fed the basal diet supplemented with 10 mg/Kg of lycopene. At the end of each experiment, broilers were slaughtered, their breasts and thighs were removed, vacuum-packed and stored at –20ºC until analyses.

The extract for the enzymatic activities analyses as well as the GSHPx activity was obtained according to the assay described by DeVore and Greene (1982). GSHPx activity was expressed as nanomoles of NADPH oxidized per minute per mg of protein.

SOD activity was measured according to Marklund and Marklund assay (1974) based on the ability of SOD to inhibit the autoxidation of pirogallol. This activity was expressed as units of SOD per mg of protein.

CAT activity was determined by measuring the spectrophotometric decrease in hydrogen peroxide at 240 nm (Mei et al. 1994).

Protein content of samples was measured by the procedure of Lowry et al (1951).

Iron-induced TBA test was carried out according the method of Kornsbrust and Mavis (1980) in order to evaluate the lipid oxidation degree of samples. Results were expressed as nmols malondialdehid (MDA)/mg tissue.

Results and discussion

The activity of the antioxidant enzymes was higher in thigh than in breast due to the different oxidative metabolism of these kinds of muscles (Tables 1 and 2).

Results from breast muscles (Table 1) showed that groups fed natural antioxidants presented higher SOD activity in relation to control group fed basal diet ($p<0.05$). No differences were noticed in CAT activity and, control and lycopene groups displayed a reduced, but not significant, GSHPx activity compared with the rest of the treatments. Statistically significant difference in TBARS levels ($p<0.05$) was obtained in samples from animals supplemented with α-tocopherol (T2) due to the protective effect of the compound. This result was not found when broilers were fed α-tocopherol plus β-carotene. This agrees with results obtained by Carreras et al. (2002) in raw breast broiler meat.

Results obtained from thighs are shown in Table 2. In this case significant differences in SOD activities were not found. GSHPx and CAT enzymes displayed similar behaviour, since carotenoid supplemented samples showed lower enzyme activities. The main differences were obtained when compared samples from T2 and T3 because of the competition between the two antioxidants. TBA obtained results, confirmed the vitamin E efficacy although higher doses were required to obtain the same effect than in breast muscles.

Arising from this work, it seemed that β-carotene and lycopene at tested doses were not enough effective to control the oxidative process proving the effectiveness of α-tocopherol supplementation.

References

Buckley, D.J.; Morrissey, P.A. Vitamin E and meat quality (1994). Proc. Nutr. Soc. 53 (2), 289-295

Carreras, I. ; Guerrero, L. ; Guàrdia, M.D.; Esteve-García, E. ; García Regueiro, J.A.; Sárraga, C.(2002) Vitamin E levels, thiobarbituric acid test and sensory evaluation of breast muscles from broilers fed α-tocopheryl acetate and β-carotene supplemented diets. J. Agric. Food Chem. (submitted)

DeVore, V.R.; Greene, B.E. (1982) Glutathione peroxidase in postrigor bovine semitendinosus muscle. J. Food Sci. 47, 1406-1409

Frankel, E.N. (1993). In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trends in Food Sci. and Techn. 4

Halliwell, B.; Murcia, M.A.; Chirico, S.; Okezie, I.; Auroma, O.I. (1995). Free radicals and antioxidants in food and in vivo: what they do and how they work. Critical Rev. in Food Sci. and Nutr. 35 (1-2), 7-20

Kornbrust, D.J.; Mavis, R.D. (1980). Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: correction with vitamin E content. Lipids, 15, 315- 322.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L., Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. J.Biol. Chem. 193, 265-275

Marklund, S.; Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a conventional assay for superoxide dismutase. Eur. J. Biochem. 47, 469-474

Mei, L.; Crum, A.D. ; Decker, E.A. (1994) Development of lipid oxidation and incubation of antioxidant enzymes in cooked porck and beef. J. Food Lipids 1, 273-283

Ruiz, J.A.; Pérez-Vendrell, A.M.; Esteve-García, E. (1999). Effect of β-carotene and vitamin E on oxidative stability in leg meat of broilers fed different supplemental fats. J. Agric. Food Chem. 47, 448-454

Stahl, W.; Sies, H. (1996). Lycopene: a biologically important carotenoid for humans?.Archives of Biochemistry and Biophysics, 336 (1) 1-9

Acknowledgements

This work was supported by INIA project SC00-005. I.Carreras recieved a FI grant from CIRIT (Generalitat de Catalunya). The authors wish to thank Narcís Sais and Irene Cordón for their technical assistance.

Table 1. Antioxidant enzyme activities and iron-induced TBA values from raw breast muscles

	GSHPx (nmols of NADPH/ min mg prot)		SOD (units of SOD/mg prot)		CAT (μmols of H_2O_2/min mg prot)		TBARS (nmols MDA/ mg breast)	
Dietary group (n=10)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
T1	4.26 ^{ab}	0.50	1.56 ^{cd}	0.50	0.61	0.47	0.143 ^{a}	0.029
T2	4.41 ^{ab}	0.74	1.96 ^{a}	0.66	0.54	0.21	0.112 ^{b}	0.043
T3	4.53 ^{a}	1.17	1.93 ^{ab}	0.61	0.47	0.25	0.131 ^{a}	0.024
T4	4.27 ^{ab}	0.80	1.94 ^{a}	0.26	0.49	0.28	0.131 ^{a}	0.033

Different superscript letters in the same column denote significant differences ($p<0.05$)

Experimental treatments: T1 basal diet (control), T2 basal diet supplemented with 30 mg/Kg of α-tocopheryl acetate, T3 basal diet supplemented with 30 mg/Kg of α-tocopheryl acetate and 15 mg/Kg of β-carotene, T4 basal diet supplemented with 10 mg/kg of lycopene.

Table 2. Antioxidant enzyme activities and iron-induced TBA values from raw thigh muscles

	GSHPx (nmols of NADPH/ min mg prot)		SOD (units of SOD/mg prot)		CAT (μmols of H_2O_2/min mg prot)		TBARS (nmols MDA/ mg thigh)	
Dietary group (n=10)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
T1	6.89 ^{abc}	0.83	2.23	0.59	1.02 ^{a}	0.27	0.125 ^{ab}	0.022
T2	7.05 ^{ab}	0.42	2.36	0.62	0.92 ^{ab}	0.24	0.117 ^{b}	0.028
T3	6.34 ^{c}	0.51	2.01	0.55	0.76 ^{c}	0.16	0.119 ^{b}	0.030
T4	6.47 ^{bc}	0.43	2.43	0.69	0.79 ^{bc}	0.14	0.128 ^{a}	0.030

Different superscript letters in the same column denote significant differences ($p<0.05$)

Experimental treatments: T1 basal diet (control), T2 basal diet supplemented with 30 mg/Kg of α-tocopheryl acetate, T3 basal diet supplemented with 30 mg/Kg of α-tocopheryl acetate and 15 mg/Kg of β-carotene, T4 basal diet supplemented with 10 mg/kg of lycopene.