Dietary lipid oxidation and vitamin E supplementation influence in vivo erythrocyte traits and postmortem leg muscle lipid oxidation in broiler chickens

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Schiavone, A., Nery, J., Choque-López, J. A., Baucells, M. D. et Barroeta, A. C. 2010. **Dietary lipid oxidation and vitamin E supplementation influence in vivo erythrocyte traits and post-mortem leg muscle lipid oxidation in broiler chickens.** Can. J. Anim. Sci. **90**: 197–202. The present work aimed to assess: (1) whether the oxidative status of dietary lipids or vitamin E supplementation influences in vivo erythrocyte integrity of chickens, and (2) whether erythrocyte stability is related to musculus iliotibialis susceptibility to lipid peroxidation. Thirty-six broilers were fed a basal diet supplemented with: sunflower oil (SO), sunflower oil and α-tocopheryl acetate (SO+E), and oxidized sunflower oil (SO-OX). In vivo hemolysis rate (HR) and thiobarbituric acid reactive substances (TBARS) of erythrocytes were measured. Postmortem, the TBARS of m. iliotibialis was determined. Erythrocyte HR and TBARS were higher in SO-OX than SO and SO+E groups ($P < 0.001$). Erythrocyte and muscle TBARS were highly correlated ($r^2 > 0.93$). The SO-OX induced negative effects, indicating that dietary lipid quality is rapidly translated in negative effects to erythrocytes and muscle. In vivo erythrocyte TBARS proved to be a good indicator of meat oxidative status.

Key words: Broiler, vitamin E, lipid oxidation, TBARS, erythrocytes, hemolysis

Schiavone, A., Nery, J., Choque-López, J. A., Baucells, M. D. et Barroeta, A. C. 2010. **L'oxydation des lipides alimentaire et la supplémentassions en vitamine E influencent in vivo les caractéristiques des érythrocytes et post-mortem l'oxydation des lipides intramusculaires chez le poulet de chair.** Can. J. Anim. Sci. **90**: 197–202. L'objectif de cette étude a été de vérifier: (1) si l'oxydation des lipides alimentaires ou la supplémentation en vitamine E influencent, *in vivo,* l'intégrité des membranes des érythrocytes de poulet, et (2) s'il y a une corrélation entre l'intégrité de la membrane des érythrocytes et la susceptibilité à la peroxydation des lipides de *Musculus iliotibialis*. Trente six poulets ont reçu un régime alimentaire de base supplémenté avec : de l'huile de tournesol (SO), de huile de tournesol et de l'α-tocophéryl acétate (SO+E) ou de l'huile de tournesol oxydée (SO-OX). *In vivo*, le taux d'hémolyse (HR) et les substances réactives à l'acide thiobarbiturique (TBARS) ont été évalués sur les érythrocytes. *Post-mortem* l'indice TBARS a été mesuré sur le *M. iliotibialis*. Les valeurs de HR et TBARS des érythrocytes ont été plus élevées pour le groupe SO-OX par rapport aux groupes SO et SO+E ($P < 0{,}001$). Les valeurs de TBARS des érythrocytes et du muscle étaient corrélées ($r^2 > 0{,}93$). Les effets négatifs du régime SO-OX suggèrent que la qualité des lipides alimentaires influence très rapidement la qualité des érythrocytes et du muscle. La valeur de TBARS des érythrocytes mesurée *in vivo*, peut être considérée comme un facteur de prédiction de la qualité de la viande.

Mots clés: Poulet, vitamine E, oxydation des lipides, TBARS, érythrocytes, hémolyse

Lipid stability of animal products during storage depends on its pro-oxidant and antioxidant content, on fat content and fatty acid profiles of fat, and on the degree of processing and the storage conditions of products (Jensen et al. 1997; Bou et al. 2009). It is well known that the quality of dietary lipid and dietary supplementation above the requirements of antioxidants leads to a significant improvement in the quality of poultry products (meat and eggs), as lipid stability during storage is enhanced (Bou et al. 2009). Furthermore, vitamin E (α-tocopheryl acetate) acts by fortifying lipid stability of cellular membranes and, in mammals, its deficiency is associated with a higher incidence of the hemolysis rate in guinea pigs (Keller et al. 2004). Erythrocytes play a major role in the physiologic functions of the circulatory system, such as oxygen transport, and their integrity is associated with welfare

Abbreviations: **ANOVA**, analysis of variance; **BHT**, butyl-hydroxy toluene; **FCR**, feed conversion ratio; **GE**, gross energy; **HR**, hemolysis rate; **MDA**, malonaldehyde; **PBS**, phosphate buffered saline; **SO**, sunflower oil; **SO+E**, sunflower oil and α-tocopheryl acetate; **SO-OX**, oxidized sunflower oil; **TBA**, thiobarbituric acid; **TBARS**, thiobarbituric acid reactive substances

conditions. Feeding oxidized oils, such as from frying waste products or sub-products from the oil refining industry, has been shown to decrease growth (Sheehy et al. 1993) and sensory traits of meat (Karpinska et al. 2001). Moreover, antioxidant supplementation of diets containing oxidized lipids was found to attenuate these negative effects (Sheehy et al. 1993).

The present work aims to verify: (1) whether the oxidative status of dietary lipids or vitamin E supplementation influences in vivo integrity of erythrocytes by measuring the hemolysis rate and susceptibility to lipid peroxidation, and (2) whether erythrocyte stability is related to musculus iliotibialis susceptibility to lipid peroxidation.

MATERIALS AND METHODS

The present study using broiler chickens was carried out in the experimental station of the Veterinary School of the Universitat Autònoma de Barcelona (UAB), Spain. The experimental protocol was approved by the Ethics Committee of the Universitat Autònoma de Barcelona. The birds were housed in accordance with European Union guidelines.

A total of 36 female broiler chickens (Ross 308) were raised under experimental conditions from the age of 21 to 35 d. Birds were randomly distributed into 18 cages (distributed into three floors; two birds/cage) and kept under controlled conditions of temperature, humidity and ventilation. Each group (six cages) was assigned to one of the treatments described below.

On a weekly basis performance traits [body weight (BW), feed consumption, and feed conversion ratio (FCR)] were evaluated. At the ages of 21 and 35 d, blood samples were taken in order to assess hemolysis rate and erythrocytes susceptibility to lipid peroxidation. After slaughtering (35 d) meat susceptibility to lipid peroxidation was measured.

Feed and water were provided ad libitum. Control of health status was carried out daily. Birds were randomly assigned to one of the three dietary treatments. The experimental diets were formulated to meet or exceed the requirements recommended by the National Research Council (1994). The diet was produced in the feed manufacturing plant of the Veterinary School of the UAB. Dietary treatments included a common basal diet supplemented with: 6% sunflower oil (SO), 6% sunflower oil + 200 mg kg^{-1} of feed α-tocopheryl acetate (SO + E), and 6% oxidized sunflower oil (SO-OX). Sunflower oil was oxidized by heating in an oven at 180 to 185°C for 22 h in order to obtain secondary products of lipid oxidation. The composition and the analyses of the diet are reported in Table 1.

Feed samples were taken for proximate analysis (Association of Official Analytical Chemists 1995) and gross energy analysis (GE) by adiabatic calorimetric bomb (IKA-calorimeter C4000®, Jankel-Kunke, Staufen, Germany). The α-tocopherol in feed samples was extracted as previously described by Jensen et al. (1999) starting from 2 g of sample. Chromatographic separation was achieved on a HS-5-Silica column (125 × 4 mm) (Perkin-Elmer, D-88662 Überlingen, Germany). The mobile phase was heptane modified with 2-propanol (99.5:0.5 vol:vol) degassed with helium. The HPLC determination was performed according to the conditions described by Jensen et al. (1999). Fluorescence detection was performed with an excitation wavelength of 290 nm and emission wavelength of 327 nm.

Sunflower oil (Koipesol®, SOS CUÉRTARA, S.A, Madrid, Spain) was used as a supplemented fat source at 6%, either in the fresh or the oxidized form. Sunflower oil was analyzed before and after the heating treatment to determine fat polymerisation: for polymer content higher than 3%, analysis was done according to International Union of Pure and Applied Chemistry (1992), and for polymer content below 3% analysis was performed according to Munster (1988). p-anisidine value was determined according to the American Oil Chemists' Society official method Cd 18-90 (1998).

Table 1. Ingredients and composition of the diet.

Ingredients (%)	SO[1]	SO + E[1]	SO-Ox[1]
Soybean meal 48% CP	32.00	32.00	32.00
Maize	30.00	30.00	30.00
Sunflower oil	6.00	6.00	–
Oxidised sunflower oil	–	–	6.00
Starch	19.80	19.80	19.80
Exal[2]	7.20	7.20	7.20
Dicalcium phosphate	2.00	2.00	2.00
Calcium carbonate	1.30	1.30	1.30
Vitamin-mineral mix[3]	1.00	1.00	1.00
Sodium chloride	0.50	0.50	0.50
DL-Methionine	0.20	0.20	0.20
Vitamin E (mg kg^{-1} of feed)	–	200.00	–
Metabolizable energy (MJ kg^{-1})[4]	12.1	12.1	12.1
Chemical analysis (%)			
Dry matter	90.9	90.9	90.9
Crude protein	19.3	19.3	19.7
Crude fat	8.3	8.0	7.9
Crude fiber	2.4	1.9	2.1
Ash content	5.8	5.8	6.0
Vitamin E (mg kg^{-1} of feed)	61.6	225.0	23.8
Gross energy (Kcal kg^{-1})	17.8	17.8	17.7
Oxidised status of oils			
polymers (%)	0.97	0.97	11.80
p-anisidine value	5.30	5.30	101.16

[1]Basal diet supplemented with: SO – 6% sunflower oil; SO + E –6% sunflower oil + 200 mg kg^{-1} α-tocopheryl acetate; SO-OX – 6% oxidised sunflower oil (SO-OX).
[2]Sepiolite (hydrous magnesium silicate).
[3]Vitamin and mineral mix per kg of feed: vitamin A: 12,000 IU; vitamin D_3: 2,400 IU; vitamin E: 30 mg; vitamin K_3: 3 mg; vitamin B_1: 2,2 mg; vitamin B_2: 8 mg; vitamin B_6: 5 mg; vitamin B_{12}: 11 μg; folic acid: 1,5 mg; biotin: 150 μg; calcium pantotenate: 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.
[4]Estimated values (NRC 1994).

At 21 and 35 d of age blood samples were collected from the brachial wing vein using heparinised collection tubes.

Erytrocythes were separated from plasma and buffy coat by centrifugation at $1000 \times g$ for 10 min at 4°C. The erythrocyte layer was washed three times with 10 volume of 10 mmol L^{-1} PBS. The washed erythrocytes were suspended in PBS and adjusted to a haematocrit of 5%. To induce lipid peroxidation, 0.6 mmol L^{-1} *tert*-butyl hydroperoxide (*tert*-BuOOH) was added to erythrocytes suspension and incubated for 90 min at 37°C in a shaking water bath (120 oscillations min^{-1}) and the extent of lipid peroxidation and hemolysis rate were measured.

The HR was determined as described by Moriguchi et al. (2001). Briefly, 200 μL of the erythrocytes suspension was added to 3.0 mL of distilled water or saline, mixed gently and centrifuged at $1500 \times g$ for 15 min at 4°C. The absorbance (Abs.) of each supernatant was measured at 540 nm. The HR was calculated according to the following equation:

$$\text{Hemolysis rate (\%)} = \text{Abs. sample/Abs. water} \times 100$$

The TBARS were measured using the method of Buege and Aust (1978). Briefly, 2.0 mL of thiobarbituric acid (TBA) solution reagent [a mixture of TBA diluted to 0.375%, 1 mol L^{-1} HCl solution diluted to 25%, trichloracetic acid diluted to 15% and butyl-hydroxy toluene (BHT) diluted to 0.4%] was added to 1.0 mL of the erythrocytes suspension, mixed vigorously and heated for 15 min in a boiling water bath. Subsequently, the samples were cooled in ice-cold water and centrifuged at $1500 \times g$ for 15 min at 4°C; the absorbance of supernatant was measured to calculate malonaldehyde (MDA) concentrations. The MDA concentration was calculated multiplying the absorbance value for the molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} (Wills 1969).

At the end of the experimental period, one bird per cage was sacrificed and m. iliotibialis was sampled, vacuum-packed and stored at –80°C until analysis. Tissue susceptibility to lipid peroxidation was evaluated by the measurement of the TBARS according to Haung and Miller (1993). Three grams of finely minced muscle were homogenized in 57 mL chilled 1.15% KCl 0.01 M phosphate buffer (pH 7.4). The homogenate (30 mL) was placed into a 50-mL conical flask, ehich was stoppered and incubated at 37°C in a shaking water bath with the addition of 8.34 mg $FeSO_4 \cdot 7H_2O$ to give a final concentration of 1.0 mM Fe^{3+} in the homogenate. At 0, 15, 30 and 60 min, 5 mL of homogenate from each flask were mixed with 2.5 mL of 15% trichloracetic acid and 0.375 mL of 0.2% BHT in absolute ethanol. The mixture was filtered through Whatman No. 1 filter paper and 4 mL of filtrate was mixed with 2 mL of 0.7% 2-TBA in a stoppered 10-mL test tube. After heating in boiling water for 15 min, the reactant was cooled and the absorbance was measured at 532 nm against a reagent blank. Malonaldehyde bis(diethyl acetal) (Aldrich Chemical Co Ltd, Dorset, UK) was used as a standard to determine the linear standard response and recovery. The TBARS values were calculated by multiplying the absorbance by a constant coefficient K (23.58) combining standard response, recovery (93.4%), molecular weight of the MDA and sample weight. The TBARS values were expressed as mg MDA kg^{-1} fresh meat.

Each cage (two birds) was considered the experimental unit for growth performance traits. The individual bird was the experimental unit for erythrocyte and muscle traits. Data were analyzed using SPSS 12.0 (Chicago, IL). The variables measured were initial and final body weights, weight gain, feed consumption, feed conversion ratio (FCR), erythrocytes TBARS, hemolysis rate, and muscle TBARS at 0, 15, 30 and 60 min. Dietary treatment was considered as the factor influencing these variables. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Repeated measures of ANOVA was used for data on muscle TBARS. Moreover, linear regressions between erythrocyte TBARS and muscle TBARS at 0, 15, 30 and 60 min were calculated. Results are presented as mean $\pm$ standard deviation (SD).

RESULTS

Birds remained healthy throughout the study, based on the absence of clinical signs. Initial and final body weights, as well as feed consumption, are presented in Table 2. The feed conversion ratio was 2.11 ± 0.23 for SO group, 2.11 ± 0.19 for SO + E group and 2.18 ± 0.17 for SO-OX group (Table 2).

Gross energy of the diet was 17.8 MJ kg^{-1} for SO and SO + E diets, and 17.7 MJ kg^{-1} for SO-OX diet. Vitamin E content of the diets was 62.0 mg kg^{-1} of feed for SO diet, 225.0 mg kg^{-1} for SO + E diet and 24.0 mg kg^{-1} for SO-OX. The sunflower oil used in the diet contained 0.97% and 11.80% of polymers before and after the heating treatment, respectively, while p-anisidine value was 5.30 and 101.16, respectively (Table 1).

The integrity of erythrocytes, evaluated as susceptibility to hemolysis and to lipid oxidation, was influenced by the dietary treatment ($P < 0.001$; Table 3). Hemolysis rate was higher in SO-OX (43.20%) than SO or SO + E groups (5.52 and 2.14%, respectively). The hemolysis rate of the SO group was also numerically higher than SO + E group. A similar trend was observed for the susceptibility to erythrocyte lipid peroxidation (Table 3). Birds fed the SO-OX dietary treatment presented a higher TBARS value (0.25 nmol MDA mL^{-1} erythrocytes) than SO (0.19 nmol mL^{-1}), while dietary treatment SO + E induced the lowest TBARS erythrocyte concentration (0.14 nmol mL^{-1}).

Table 2. Chickens growth performance traits ($n=6$) (mean ± SD)

	SO[z]	SO + E[z]	SO-Ox[z]
Initial body weight (day 21) (g)	879.6 ± 80.3	884.3 ± 14.3	870.9 ± 84.0
Final body weight (day 35) (g)	1530.4 ± 83.0	1525.7 ± 73.3	1478.6 ± 89.4
Weight gain (days 21–35) (g)	592.4 ± 87.8	583.1 ± 89.9	591.0 ± 104.7
Feed consumption (day 21–35) (g)	1371.6 ± 122.6	1341.2 ± 77.2	1323.1 ± 127.3
FCR (days 21–35)	2.11 ± 0.23	2.11 ± 0.19	2.18 ± 0.17

[z]Basal diet supplemented with: SO, 6% sunflower oil; SO + E, 6% sunflower oil + 200 mg kg^{-1} α-tocopheryl acetate; SO-OX, 6% oxidised sunflower oil (SO-OX).

The TBARS values of m. iliotibialis are presented in Fig. 1. Overall mean differences between treatments were reflected throughout the induced lipid peroxidation ($P<0.001$): TBARS muscle concentrations were 5.10, 1.81, and 6.69 mg kg^{-1} meat for SO, SO + E and SO-OX, respectively (mean values from Fig. 1). At 0 min after induction of tissue lipid peroxidation, the SO-OX dietary treatment induced the highest TBARS muscle concentration (2.69 mg kg^{-1} meat), compared with the lower values of SO and SO + E treatments (2.05 and 1.23 mg kg^{-1} meat). The same was observed after 15, 30 and 60 min. Increasing concentrations of TBARS in SO and SO-OX presented a similar pattern over time following peroxidation induction, while SO + E treatment produced a lower increase rate of TBARS concentration.

Correlation between erythrocyte and muscle TBARS values are presented in Table 4. Positive linear relationships between TBARS values were found in the present study ($r^2=0.96$ at 0 min following peroxidation induction; $P<0.001$). The correlation was also observed for muscle peroxidation following 15, 30 and 60 min of peroxidation induction.

DISCUSSION

In contrast to most other studies dealing with the effects of thermoxidized fats (Corcos Benedetti et al. 1987; Dibner et al. 1996; Engberg et al. 1996; Hochgraf et al. 1997), feeding a diet containing oxidized sunflower oils did not impair growth and did not cause symptoms of toxicity, but the dietary treatment in our experiment lasted only 14 d. The results obtained were in accordance with the observations of Keller et al. (2004) in rats, of Oertel (1982) in guinea pigs and chickens, and Sheehy et al. (1993) in chickens fed sunflower oil, but not linseed oil. In general, growth performance traits were not affected by the supplementation with α-tocopheryl acetate. Similar results were observed by Allen and Fetterer (2002), whose study showed that increasing concentrations of DL-α-tocopheryl acetate did not promote growth or FCR in either group of healthy chicks and animals infected with *Eimeria maxima*. This means that the effects observed on erythrocytes integrity and susceptibility to lipid oxidation were not confounded by secondary effects of reduced growth.

Higher hemolysis rate was observed when birds were fed oxidized fat sources (SO-OX). This is in accordance with previous observations concerning the hemolysis rate in guinea pigs fed either fresh or oxidized fat with increasing concentrations of vitamins E and C (Keller et al. 2004). The authors suggested that higher hemolysis susceptibility was due to a deficiency of antioxidant defences. In the present study, the highest hemolysis rate could therefore be related to the pro-oxidative action of the SO-OX treatment against the cellular membrane. Moreover the supplementation of vitamin E (SO + E) induced the lowest susceptibility to hemolysis, which was due to an improvement of antioxidant defences of erythrocytes. This was in accordance with the observations of Soto-Salanova and Sell (1996). The authors have found that supplementation with α-tocopherol (80 and 150 IU) reduced erythrocyte susceptibility to hemolysis in turkeys. Previous studies have stated that vitamin E and selenium supplementation in chickens induce a higher superoxide dismutase concentration in erythrocytes, supporting that dietary supplementation with antioxidants leads to an improvement of erythrocytes oxidative status (Traş et al. 2000). The higher susceptibility to

Table 3. Erythrocytes traits ($n=5$) (mean ± SD)

		SO[z]	SO + E[z]	SO-Ox[z]
Day 21	TBARS[y]	0.22 ± 0.01	0.21 ± 0.02	0.20 ± 0.03
	Haemolysis rate (%)	18.50 ± 0.51	19.23 ± 1.26	18.52 ± 1.42
Day 35	TBARS[y]	0.19*b* ± 0.02	0.14*c* ± 0.01	0.25*a* ± 0.02
	Haemolysis rate (%)	5.52*b* ± 1.44	2.14*b* ± 0.97	43.20*a* ± 5.10

[z]Basal diet supplemented with: SO, 6% sunflower oil; SO + E, 6% sunflower oil + 200 mg kg^{-1} α-tocopheryl acetate; SO-OX, 6% oxidised sunflower oil (SO-OX).
[y]Malondialdehyde concentration in nmol mL^{-1} erythrocytes suspension.
a–c Within a line, means without common superscript differ ($P<0.001$).

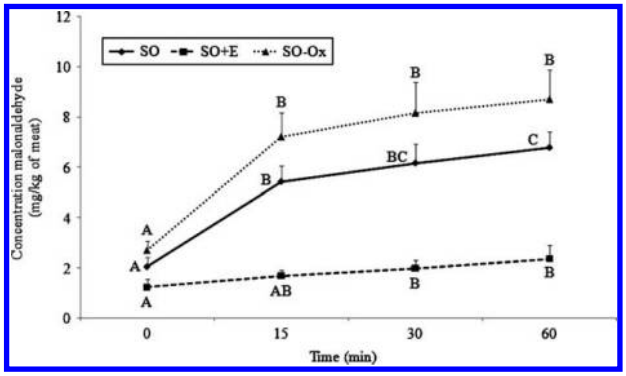

Fig. 1. Concentration of malonaldehyde in the m. iliotibialis. Basal diet supplemented with: SO, 6% sunflower oil; SO+E, 6% sunflower oil+200 mg kg^{-1} α-tocopheryl acetate; SO-OX, 6% oxidised sunflower oil (SO-OX); muscle peroxidation following 15, 30 and 60 min of peroxidation induction. Data presented as mean±SD. A, B For each group means without common letter differ ($P<0.001$).

hemolysis when chickens were fed SO compared with SO+E may be associated with the richness of polyunsaturated fatty acids of the SO diet, which could be transferred in the cellular membrane and would not be protected by sufficient amounts of vitamin E.

Vitamin E acts as a strong antioxidant, improving lipid stability. Higher TBARS were previously found (Sheehy et al. 1993) in chickens fed heated sunflower oil (either supplemented or not with α-tocopheryl acetate). The results obtained support that the susceptibility to hemolysis could be related to erythrocyte oxidative status. This is due to higher erythrocyte susceptibility to peroxidation that was found when chickens were fed diets with higher pro-oxidant and/or lower antioxidant content. Results suggest therefore that erythrocyte traits are influenced by dietary treatments and that oxidized oils are not adequate in maintaining erythrocyte integrity. It is advisable that these parameters would be taken into account as predictors of the quality of poultry products. The negative effects on the structure of the erythrocytes induced by oxidized sunflower oil suggest that the quality of feed (in particular of lipids) is rapidly converted in negative effects to the organism.

Results on muscle TBARS concentration indicated that feeding oxidized fat sources led to higher values than feeding fresh or vitamin E supplemented fats after induction of peroxidation. This was in accordance with the observations of Sheehy et al. (1993), but the authors found a steady increase of TBARS in thigh meat of chickens fed fresh sunflower oil, which was similar to the increase observed in the present study for SO+E. Despite the different pattern observed for fresh oil, the results obtained concerning SO-OX were similar in both studies. Meat storage stability has previously been reported to be lower in chickens fed oxidized compared with fresh oils (Galvin et al. 1997; Jensen et al. 1997). The higher susceptibility of SO-OX compared with SO indicates that meat lipid peroxidation of chickens fed oxidized lipids is likely to be higher through storage processes than in birds fed fresh sunflower oil. Furthermore, the present study indicates that vitamin E supplementation could be an adequate nutritional strategy to maintain meat lipid stability during storage because it decreases muscle susceptibility to lipid peroxidation. To attain this objective, previous studies (Galvin et al. 1997; Jensens et al. 1998) recommend a dietary supplementation of α-tocopherol of 200 mg kg^{-1} feed in broiler diets. The present study supports that this dietary concentration is adequate to maintain lipid stability of broiler meat.

When feeding different levels of polyunsaturated fatty acids to broilers and rats [Febél et al. (2008) and Sarkadi-Nagy et al. (2003), respectively], higher susceptibility of dietary fat to peroxidation would lead to higher susceptibility of body tissues to peroxidation. Moreover, previous authors observed that feeding oxidized fat to broilers (Engberg et al. 1996) and rats (Suzuki et al. 1999) would influence positively erythrocyte TBARS. This influence of diet, in particular for oxidized fat sources, was confirmed in the present study since feeding oxidized sunflower oil led to significantly higher erythrocyte TBARS, together with muscle TBARS. In the past few years, the effect of diet on both variables has been widely studied but, to our knowledge, no correlation between erythrocyte and muscle TBARS has yet been shown. Given the results obtained in the present study concerning the positive linear correlation between erythrocyte and muscle TBARS values, the oxidative status of erythrocytes is reflected in the oxidative status of other tissues, such as m. iliotibialis. Erythrocyte susceptibility to lipid peroxidation constitutes, therefore, a reliable predictor of lipid stability in meat during storage. This could be a useful tool to assess muscle membrane susceptibility to peroxidation during the fattening period of broilers.

In conclusion, in this study, feeding oxidized sunflower oil increased the susceptibility of erythrocytes and muscles to lipid peroxidation. Therefore, α-tocopheryl acetate supplementation is an adequate nutritional strategy to improve both erythrocyte integrity and meat shelf-life. Erythrocyte and muscle susceptibility to lipid peroxidation are closely correlated, and this could represent an interesting predictive measure concerning

Table 4. Correlation between erythrocyte and m. iliotibialis TBARS

	r^2	P value	SE
$y_0=10.368x^z$	0.96	<0.001	0.44
$y_{15}=25.836x$	0.93	<0.001	1.49
$y_{30}=29.413x$	0.93	<0.001	1.65
$y_{60}=31.925x$	0.94	<0.001	1.70

$^z x$, erythrocyte TBARS (nmol mL^{-1}); y_t, m. iliotibialis TBARS (mg kg^{-1} of meat) at 0, 15, 30 and 60 min after m. iliotibialis peroxidation induction.

lipid stability during the storage of meat. The utilisation of waste products might be financially convenient, but severe consequences of feeding these products to chickens are reflected in lipid conservation during storage.

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