



Original Research Article

Effect of age and different doses of dietary vitamin E on breast meat qualitative characteristics of finishing broilers



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ABSTRACT

The supplementation of vitamin E on broiler chicken diets is essential to the prevention of lipid oxidation reactions in the meat and improvement of meat quality. The objective of this study was to assess the effect of different doses of dietary vitamin E on breast meat quality of broiler chickens in the finishing period. Five doses of vitamin E were used (30, 90, 150, 210, and 270 mg/kg feed) in broilers' diets from 42 to 54 d of age. A completely randomized design was conducted, followed by a split-plot, where the vitamin E dose was considered as the whole plot, and broilers' age at slaughter was the subplot. Breast meat quality was assessed at 4 different ages (45, 48, 51, and 54 d old), using 50 birds per age, totaling 200 birds. Meat quality characteristics evaluated were: pH at 24 h post mortem, color (brightness, redness, and yellowness), water holding capacity, cooking loss, shear force, and lipid peroxidation. There was no interaction between age and dose of vitamin E for meat quality characteristics ($P > 0.05$). The age at slaughter had a quadratic effect ($P < 0.05$) on pH, brightness, redness, and water holding capacity. Although pH values were higher in the breast meat of older birds (51 and 52 d old), breast meat of younger birds (48 d) had a more reddish aspect. Shear force value was higher in breast meat of birds slaughtered at later ages ($P < 0.01$), as a linear age-effect was observed. Brightness increased linearly ($P < 0.05$) with higher vitamin doses, whereas treatments did not alter yellowness, cooking loss, and lipid peroxidation. In this study, increasing vitamin E doses in the finishing period increased the brightness of broiler breast meat, whereas slaughtering at later ages resulted in greater meat pH and shear force value.

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1. Introduction

In poultry farming, there are many stressing factors that influence the growth and productive performance of broiler chickens, such as thermal and nutritional stress, overcrowding, invasive procedures like vaccination and pecking (Selvam et al., 2017), pre-slaughter handling, transportation and slaughter (Petracci et al.,

2010). Carcass yield and meat quality, for example, are directly affected by these stressing factors, but formulation strategies regarding the use of antioxidants and supplementation of Vitamin E on broiler diets can be applied in order to improve the chicken immune system and improve meat quality (Barroeta, 2007; Lu et al., 2014).

The levels of vitamin inclusion in broiler chicken diets can be very divergent. In Brazil, vitamin requirements commonly used are listed in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011) and, as in other countries, many diets are also formulated according to the NRC (1994). On these references, the vitamin levels displayed are the minimum requirements through which good growth rates can be achieved for broiler chickens, but there is an inadequate correlation between the Brazilian Tables and the NRC, and the industry recommendations for vitamin dietary inclusion. According to Félix et al. (2009),

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variations in the supplemented levels of fat-soluble vitamins (A, D, K, and E), for example, can be up to 24 times higher in commercial diets compared to those recommended by industry references.

Vitamin E is one of the essential vitamins in animal nutrition, and is usually provided to monogastric animals in the form of synthetic alpha-tocopherol. Although there are other tocopherols (beta, gamma, and delta-tocopherol) and 4 tocotrienols (alfa, beta, gamma and delta-tocotrienol), alpha-tocopherol is the most biologically effective form of vitamin E (Halliwell and Gutteridge, 1999), because of its more efficient antioxidant activity in cells (Surai, 2003). Vitamin E supplementation is an effective way to prevent lipid oxidation reactions in the meat, a process that leads to the formation of toxic compounds that adversely interfere with meat quality, often associated with discoloration, unpleasant taste and odor (Jensen et al., 1998; Nam et al., 2002; Wang et al., 2009).

The NRC (1994) recommends a vitamin E dose of 10 mg/kg of broiler chickens fed during the entire rearing period, and the Brazilian Tables' recommended levels range from 35 mg/kg (pre-starter period) to 18 mg/kg (finishing period). Other studies recommend greater vitamin E levels, ranging from 200 to 500 mg/kg in broiler starter diets, to achieve optimum health and immune responses (Chung and Boren, 1999) and meat quality (Zhang et al., 2011; Hashizawa et al., 2013). Increasing levels of dietary vitamin E for broilers at an early age can be costly, thus raising the matter of determining whether vitamin E levels for broiler chickens could be increased only during the finishing period, in an attempt to improve meat quality parameters pre-slaughtering while lowering vitamin supplementation costs. Therefore, the objective of the present study was to assess the effect of different doses of vitamin E on breast meat quality of broiler chickens in the finishing period.

2. Materials and methods

2.1. Broiler chickens and management

Research on animals was approved by the Ethics Committee on the Use of Animals, Federal University of Paraná, Agricultural Sciences Sector, under protocol number 009/2015. The experiment was conducted in Curitiba, Brazil (25°25'40"S and 49°16'23"W). A total of 750 Cobb 500 (Cobb Brazil Ltda, São Paulo, Brazil) male broiler chickens were used from 42 to 54 d. The birds were distributed in 50 pens of 2.06 m² each (15 birds/pen), equipped with tubular feeders, nipple drinkers, heating resistors and wood shavings for litter. Birds received feed and water ad libitum and were raised under experimental conditions in order to minimize stress during the rearing period. Temperature was set at 18 °C on the first week and reduced to 17 °C on the second week.

2.2. Experimental design and diets

A completely randomized design was conducted, followed by a split-plot in time design with 5 treatments and 10 replicates of 15 birds each at 4 different ages at slaughter. Treatments varied according to dietary levels of Vitamin E (30, 90, 150, 210 and 270 mg/kg feed), and each bird was considered an experimental unit for each slaughter age. From 1 to 42 d, birds were properly fed with initial, growth and finisher diets, formulated according to the Brazilian Tables for Poultry and Swine (Rostagno et al., 2011) where all treatments received the same basal diet containing 100 mg vitamin E and 200 µg selenium per kilogram of feed. During the experimental period (42 to 54 d old), each treatment received the proper experimental diet (Table 1). High performance liquid chromatography

Table 1

Ingredients and nutrient composition of the experimental diet (42 to 54 d).

Item	Content
Ingredients, g/kg (as-fed basis)	
Corn	672.0
Soybean meal	264.5
Soybean oil	37.3
Dicalcium phosphate	9.9
Calcitic limestone	6.4
Common salt	3.8
Vitamin-mineral supplement ¹	0.9
l-lysine	2.4
DL-methionine	2.2
l-threonine	0.6
Calculated composition, g/kg (dry matter basis)	
Crude protein	176.0
Calcium	6.1
Chlorine	2.6
Sodium	1.9
Potassium	6.4
Available phosphorus	2.8
Digestible methionine + Cystine	7.3
Digestible methionine	4.8
Digestible lysine	10.0
Digestible threonine	6.5
Metabolizable energy, kcal/kg	3,250

¹ Vitamin-mineral supplement formulated without vitamin E. It provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D₃, 3,600 IU; vitamin K₃, 3.15 mg; vitamin B₁, 2.25 mg; vitamin B₂, 5.4 mg; vitamin B₆, 3.6 mg; vitamin B₁₂, 18 mg; niacin, 54 mg; pantothenic acid, 9 mg; biotin, 0.18 mg; manganese, 63 mg; iron, 45 mg; copper, 9 mg; zinc, 63 mg; iodine, 1.08 mg; selenium, 135 mg.

(HPLC) was used to determine the final concentrations of vitamin E in the experimental diets.

2.3. Breast meat samples collection

At 45, 48, 51 and 54 d old, 10 birds per treatment were randomly selected and underwent an 8 h period of feed restriction before slaughter. The birds were then identified and transported to the slaughter plant, individually weighed and stunned before the euthanasia. After the euthanasia, the carcasses were cooled by immersion in a water and ice bath at 0 to 2 °C for 60 min. After cooling, the whole breast muscle with bone was severed from the carcass, placed in duly identified plastic bags and stored at 4 °C for 24 h. After 24 h, the breast was deboned and muscle samples were collected from both sides of the breast and stored at 4 °C for 24 h before the analyses. The right-side portion was used to analyze the meat quality variables (pH, color, water holding capacity, cooking loss, and shear force) and the left portion was frozen at -15 °C for 45 d for the lipid oxidation analysis.

2.4. pH and meat color measurement

The pH of the breast meat was determined at 24 h postmortem by using a potentiometer (Testo 205 pHmeter, Testo SE & Co., São Paulo, Brazil) inserted 2.5 cm deep in the cranial portion of the *Pectoralis major* muscle, according to the method described by Boulianne and King (1995). A portable colorimeter (Chroma CR-10, Konica Minolta Sensing, Tokyo, Japan) was used to express the CIE (International Commission on Illumination) values for meat brightness (L*), redness (a*) and yellowness (b*). Three readings were taken at 3 distinct areas in the dorsal side of the muscle, free from defects such as bruises, discolorations, and picking damage that could affect the color reading. Mean values of L*, a* and b* were then calculated.

2.5. Water holding capacity measurement

To measure the water holding capacity (WHC), a sample (2 ± 0.10 g) was obtained from the cranial portion of the breast and weighed according to the method described by (Hamm (1959)). Duplicate samples were placed on filter paper on 2 acrylic plates and maintained under a 10-kg weight for 5 min. The samples were then weighed again and the WHC value was determined by the following equation: $WHC (\%) = 100 - [(Initial\ weight - Final\ weight)/Initial\ weight] \times 100$.

2.6. Cooking loss and shear force measurement

Breast meat samples were weighed, packaged in a tightly sealed plastic bag, and cooked for 30 min in a water-bath at 85 °C. After being cooked, samples were cooled to ambient temperature by running water and weighed again to determine the cooking loss (CL) as a percentage of the weight before and after cooling (wet basis), as described by Cason et al. (1997). The same samples were then subsequently cut into 1.5 cm wide and 1.0 cm deep fillets and used in the shear force (SF) test conducted to determine meat tenderness, by using a Warner-Bratzler shear attachment mounted on a CT3 Texture Analyzer (Brookfield Engineering Laboratories, Inc., Middleboro, US). The equipment was previously calibrated with a 5-kg load cell and a blade descent speed of 5 mm/s. SF was measured on the ventral side of the fillets and the razor blade penetration was perpendicular to the muscle fibers. SF value was considered as the peak of the shear force profile.

2.7. Lipid oxidation measurement

Lipid oxidation in the breast meat samples was determined by quantifying malonaldehyde values, using the thiobarbituric acid (TBARS) reactive compounds methodology described by Pikul et al. (1989). A sample (10 ± 0.1 g) was processed in an Ultra Turrax homogenizer with 20 mL of trichloric acid. Thiobarbituric acid (5 mL) was then added to 5 mL of the filtrate and placed in a water-bath at 85 °C for 35 min. After samples were cooled, the reading was performed in a 530-nm spectrophotometer and results were expressed as malonaldehyde milligrams per kg of sample.

2.8. Statistical analysis

All the collected data was submitted to a Shapiro–Wilk normality test and, after the initial assumptions were met, an analysis of variance was performed. Data with normal distribution were analyzed using the GLM procedure of the SAS statistical package (version 8, SAS Institute Inc., Cary, NC, USA), where each bird was the experimental unit, according to the statistical model: $Y_{ijk} = \mu + \alpha_i + \delta_{jk} + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the value observed in the experimental unit that received level i of factor A (doses of vitamin E = 30, 90, 150, 210 and 270 mg/kg of feed), with level j of factor B (age of birds at slaughter = 45, 48, 51 and 54 d) in repetition k ; μ is the mean of the observed variable; α_i is the effect of the i th factor A; δ_{jk} is the experimental error of factor A within plots; β_j is the effect of the j th of factor B; $\alpha\beta_{ij}$ is the interaction of factors A and B and ε_{ijk} is the experimental error of factor B within plots. In the effect of graded age at slaughter and levels of vitamin E, the data were submitted to linear and quadratic regression analyses at 5% significance.

3. Results

The vitamin E concentration values in the experimental feeds were established as 30, 90, 150, 210, and 270 mg/kg feed, and the

analyzed values found were 21, 90, 127, 190, and 244 mg/kg feed, respectively.

Under the conditions in which the present study was conducted, no interaction between age at slaughter and vitamin E dose were found ($P > 0.05$) for the evaluated meat quality variables (Table 2). When looking at the main effects, the variables pH, a^* , WHC, SF, and TBARS were only influenced by the age at slaughter ($P < 0.05$). Only the L^* variable significantly differed at both the age at slaughter and vitamin E doses. There were no significant differences in b^* and CL ($P > 0.05$).

Results for the analyses of regression are shown in Table 3. Considering the age factor, a quadratic effect was observed on L^* , which was higher at 45 and 51 d of age; in relation to vitamin E dose, L^* had a linear increase (P -linear < 0.01) with higher doses. Age at slaughter had a quadratic effect on pH (P -quad < 0.01), a^* (P -quad < 0.01) and WHC (P -quad < 0.01) and a linear effect was observed on SF (P -linear < 0.01). Breast meat of older broilers had higher values of pH (at 51 and 54 d) and SF (at 54 d) compared to those slaughtered at 45 and 48 d, whereas a^* and TBARS were higher on breast meat of birds at 48 d, and WHC was higher on birds slaughtered at 48 and 51 d.

4. Discussion

Supplementation with different levels of vitamin E did not have an effect on the pH values of broiler breast meat, but was rather influenced by the birds' age at slaughter, as pH was higher on older birds. A study by Baéza et al. (2012) also reported a similar influence of increasing age at slaughter (35 and 49 d old) on meat pH of broilers, as older birds had higher pH values. The authors state that the observed changes in pH are related to changes in lactate and glycolytic potential, both processes that cause a reduction in the muscle pH and both higher in younger birds.

Although meat pH results at 24 h postmortem were obtained every 3 d during an experimental period of 12 d, similar results were found by Leonel et al. (2007) when feeding broilers a diet supplemented with 300 mg vitamin E per kg of feed during 5 different periods (1 to 15, 1 to 30, 1 to 45, 15 to 45 and 30 to 45 d of age), and by Souza et al. (2006) testing different vitamin E levels (0, 100, 150, and 200 mg/kg feed) throughout a 49-d production period. These studies also demonstrated that vitamin E supplementation had no significant effect on the final meat pH in the evaluated periods, indicating that changes in pH are more intimately related to the birds age, weight, and growth rate.

Meat color results obtained in this study showed that only L^* was influenced by dietary vitamin E levels, as it linearly increased with increasing vitamin E doses. This can be seen as a controversial result, as studies report that selenium or vitamin E supplementation can actually limit an unwanted meat discoloration by reducing the cellular damage caused by oxidation processes (Wang et al., 2009; Puvaca and Stanacev, 2011). Olivo et al. (2001) observed, for example, that vitamin E supplementation successfully prevented negative effects of heat stress on increasing L^* and decreasing a^* and b^* of broiler meat, whereas in the current study vitamin E increased L^* value of meat under normal temperature. Leonel et al. (2007), on the other hand, showed that vitamin E supplementation had no significant effect on a^* and b^* variables of broiler meat, similar to the current study, and Zhang et al. (2011) reported that vitamin E did not affect broiler meat color, which is apparently much more influenced by changes in pH. Regarding age effects on meat color, the meat of birds slaughtered at 48 d had a more reddish aspect (higher a^*) compared to older birds (51 and 54 d old). These findings differ from those by Bianchi et al. (2006), who states that older broilers presents a more reddish and darker aspect to the meat because of a greater amount of circulating

Table 2

Quality parameters of breast meat from broiler chickens supplemented with different vitamin E doses and slaughtered at 45, 48, 51, and 54 d of age.

Item	pH ¹	L*	a*	b*	WHC, %	CL, %	SF, N	TBARS, mg/kg
Age at slaughter, d								
45	5.84	53.08	0.03	11.63	67.72	27.74	18.08	0.225
48	5.94	52.93	0.53	11.56	70.62	29.18	18.01	0.371
51	6.09	53.90	0.12	11.57	70.69	28.00	22.66	0.250
54	6.05	52.14	0.04	12.25	69.54	29.38	26.37	0.329
SEM	0.019	0.364	0.107	0.189	0.573	0.602	0.965	0.025
Vitamin E dose ² , mg/kg								
30	6.00	52.57	-0.07	11.61	69.98	27.66	21.24	0.290
90	5.94	52.75	0.32	11.59	68.94	28.34	20.33	0.302
150	6.01	52.69	0.25	11.95	70.68	29.32	20.74	0.291
210	5.99	52.73	0.32	11.52	69.76	27.95	22.08	0.317
270	5.95	54.31	0.10	12.10	68.84	29.59	21.28	0.270
SEM	0.022	0.407	0.120	0.211	0.641	0.673	1.079	0.028
P-value								
Age (A)	<0.001	0.007	0.002	0.354	0.008	0.267	<0.001	0.001
Dose (D)	0.086	0.017	0.155	0.143	0.398	0.134	0.597	0.283
A × D	0.588	0.639	0.102	0.568	0.0631	0.486	0.799	0.978

L* = brightness; a* = red intensity; b* = yellow intensity; WHC = water holding capacity; CL = cooking losses; SF = shearing force; TBARS = thiobarbituric acid.

¹ pH 24 h after slaughter.² Estimated values of vitamin E concentration. Analyzed concentrations were 21, 90, 127, 190 and 244 mg/kg feed.**Table 3**P-value, regression models and determination coefficient (R²) for quality variables of breast meat from broilers supplemented with different vitamin E doses and slaughtered at 45, 48, 51, and 54 d of age.

Item	P-value		Regression models	R ²
	Linear	Quadratic		
Effect of age				
pH ¹	<0.001	0.003	pH = -0.004 × Days ² + 0.4238 × Days - 5.1403	0.9246
L*	0.261	0.031	L* = 0.0448 × Days ² + 4.3763 × Days - 53.251	0.5250
a*	0.436	0.009	a* = 0.0162 × Days ² + 1.5927 × Days - 37.734	0.5429
WHC	0.033	<0.001	WHC = 0.1122 × Days ² + 11.291 × Days - 213.09	0.9775
SF	<0.001	0.060	SF = 0.984 × Days - 27.428	0.8939
Effect of vitamin dose				
L*	0.010	0.070	L = 0.0057 × dose + 52.17	0.5610

L* = brightness; a* = red intensity; WHC = water holding capacity; CL = cooking losses; SF = shearing force; TBARS = thiobarbituric acid.

¹ pH 24 h after slaughter.

myoglobin, compared to younger birds. Another curious observation is that, according to Mercier et al. (1998), an higher pH can lead to a darker coloration of the meat (i.e. increased a* values), but in the current study this was not true, as the meat of birds slaughtered at later ages had higher pH but lower a* values.

WHC is an indication of the meat's capacity to retain water within the muscle structure and is also linked to CL results. According to Cheah et al. (1995), vitamin E supplementation can improve WHC through the inhibition of the A2 phospholipase enzyme activity, a calcium-dependent enzyme responsible for phospholipid cleavage on the cell membrane, which leads to an instability of the membrane and affects the cell capacity to hold its inherent moisture. In the current study, however, vitamin E had no significant effect on WHC and CL. Zhang et al. (2011) also observed no difference in WHC of meat, recorded as drip loss, between broilers fed a 500 IU vitamin E-supplemented diet and those fed a control diet. According to the authors, the effects of vitamin E on WHC of meat are actually limited, and the results can be inconsistent due to different levels of vitamin E supplementation and muscle compositions. Although age at slaughter had no effect on CL, the breast meat of older birds (48, 51, and 54 d) had a slightly higher WHC than that of younger birds (45 d), which can be explained by age-related changes in broiler meat ability to hold water (Northcutt et al., 1994).

SF indicates the amount of force necessary to rupture a certain muscle area, and in the current study, SF values were higher in birds at later ages. These results are in agreement with reports by

Castellini et al. (2002), who evaluated meat quality of broilers raised in a conventional system and slaughtered at different ages (56 and 81 d) and observed that SF was higher in the meat of older birds. The linear age-related effect on SF values can also be compared with data from Bertram et al. (2007). The authors conducted a study with swine and observed a more homogeneous distribution of the myofibrillar water in the meat of younger animals (90 d old) compared to older animals (140 and 180 d old). Fang et al. (1999) also explained this age effect on SF by stating that older animals have a higher proportion of connective tissue surrounding the muscle, which can be associated with an increased muscle resistance to rupture.

Similar to the results of this study, Boschini (2011) did not report any effect of vitamin E supplementation on TBARS. Leonel et al. (2007), however, assessed TBARS value in breast meat (stored under refrigeration for 3 d) of broilers supplemented with 150 mg vitamin E per kg of feed during different periods, and observed a positive effect of vitamin E on TBARS of leg and breast muscle after 3 d storage. The authors state that the antioxidant effects of vitamin E can successfully conserve meat products during short periods of storage, and this reduction on peroxidation through vitamin E supplementation is also addressed by other studies (Wang et al., 2009; Puvaca and Stanacev, 2011). In the current study, TBARS values in broiler breast meat after being stored in the freezer for 45 d suggest that the vitamin dose that was used up to 42 d of age was sufficient to keep TBARS at a low level.

5. Conclusion

The present study concludes that the different supplemented vitamin E doses had little effect on qualitative characteristics of broiler breast meat, as it only affected meat brightness, whereas meat quality was more significantly affected by the age at slaughter. Water holding capacity, pH, and shear force values were higher on breast meat of broiler chickens slaughtered at later ages (>48 d).

Author contributions

Vivian Vieira: investigation, writing – original draft; Francielle O. Marx: formal analysis; Lucas S. Bassi: writing – review & editing; Marley C. Santos: data curation; Alexandre Oba: resources, methodology; Simone G. Oliveira: supervision, visualization; Alex Maiorka: conceptualization, validation.

Conflict of interest

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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