Effects of vitamin E, an oil blend and L-Arginine on breast meat from broiler chickens

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Abstract

The study examined the effects of supplementing vitamin E on the fatty acid profile and breast meat quality of broilers fed diets containing an oil blend and L-Arginine. Two hundred sixteen Cobb 500 one-day-old broilers were randomly allocated to six treatments, namely T1: 6% palm oil (control); T2: blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine (positive control); T3: T2 with 20 mg/kg vitamin E added; T4: T2 with 50 mg/kg vitamin E added; T5: T2 with 100 mg/kg vitamin E added; and T6: T2 supplemented with 150 mg/kg vitamin E. Relative to T1, the other diets increased growth rate and improved feed conversion ratio (FCR) similarly. Linoleic, arachidonic, and polyunsaturated fatty acids were greater and palmitic and saturated fatty acids were lower in the meat of birds fed T2–T6 relative to T1. Supplementation of vitamin E reduced drip loss (DL) and increased redness, tenderness, free thiol content, and the oxidative stability of meat during storage at 4 °C. Regardless of diet, free thiol, redness and Warner-Bratzler shear force (WBSF) value decreased significantly, whereas the carbonyl content, thiobarbituric acid-reactive substances (TBARS), and DL of breast meat increased over the ageing period. In conclusion, relative to T1, the other diets were effective in improving growth performance and meat quality during post-mortem storage.

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Introduction

Dietary fat and oils are often added to the diet of rapidly growing birds to fulfil their energy requirements and thus improve their productivity at low cost (Abdulla *et al.*, 2015). Diets that contain unsaturated fatty acids (UFAs) have greater metabolizable energy values owing to better intestinal absorption compared with those that contain saturated fats (Tancharoenrat *et al.*, 2012). Further, a high intake of saturated fatty acids (SFAs) and cholesterol is associated with increased occurrence of human diseases, especially cardiovascular disease (Houston *et al.*, 2011). Therefore, supplementation of unsaturated fats could be used to alter the fatty acid profile of chicken meat to make it a functional food. Health benefits would be anticipated from feeding diets that contained a combination of palm oil (PO) and sunflower oil (SO), which would lead to increased polyunsaturated fatty acids (PUFAs) and decreased fat and cholesterol contents in the meat (Khatun *et al.*, 2017).

Feeding unsaturated fats to poultry, particularly PUFAs, can enhance lipid oxidation and affect meat flavour and colour adversely (Abdulla *et al.*, 2015), reduce performance (Zhong *et al.*, 2014), and induce oxidative challenges that affect their health. These harmful effects can be overcome with supplemental antioxidant such as vitamin E, which may also increase the shelf life of the meat (Li *et al.*, 2009).

L-Arginine is an essential amino acid for chickens and has an active role in protein synthesis (Tan *et al.* 2009), tissue growth, and enhancement of immune response (Emadi *et al.*, 2010). It has also been found to be effective in reducing body fat in chickens (Fouad *et al.*, 2013; Khatun *et al.*, 2019). Thus, it was

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hypothesized that supplementation of vitamin E to diets that contained a blend of PO and SO and L-Arginine would increase growth performance, have favourable effects on the fatty acid profile of breast muscle, improve meat quality, and increase shelf life. The objective of this study was to evaluate the effects of feeding an oil blend, L-Arginine, and vitamin E on the fatty acid profile, meat quality, lipid and protein oxidation of breast muscle of broiler chickens.

Materials and Methods

The experiment followed the guidelines sanctioned by the Institutional Animal Care and Use Committee of Research Policy at Universiti Putra Malaysia (UPM/IACUC/AUP-R081/2016). Two hundred and sixteen Cobb 500 one-day-old broiler chicks were randomly assigned to one of six treatments in a completely randomized design. Each chick was weighed and wing-banded. There were six replicates of each treatment with six chicks per replication. The experimental diets consisted of T1: 6% palm oil (control); T2: a blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine (positive control); T3: T2+ 20 mg/kg vitamin E; T4: T2 + 50 mg/kg vitamin E: T5: T2 + 100 mg/kg vitamin E; and T6: T2 + 150 mg/kg vitamin E. Arginine was added in the form of L-Arginine monohydrochloride (99.6% L-Arg) (HerbstoreUSA,Walnut, California, USA) and vitamin E as α-tocopherol acetate (Adisseo Asia Pacific Pte., Ltd., Singapore). All diets were iso-caloric and iso-nitrogenous and wereformulated according to the nutrient requirements for broilers using FeedLIVE software (FeedLIVE 1.52, Bangkok, Thailand). The feeding trial lasted six weeks. The starter diets (Table 1) were offered on days 1 to 21. Their nutritional composition is presented in Table 2. The finisher diets (Table 3) were offered on days 22 to 42. Their nutritional composition is presented in Table 4.

Table 1 Ingredients of experimental starter diets for broilers

Ingredient, g/kg	Dietary treatment					
	T1	T2	T3	T4	T5	T6
Corn	441.80	435.10	435.10	435.10	435.10	435.10
Soybean meal	312.40	329.70	329.70	329.70	329.70	329.70
Palm oil	60.00	40.00	40.00	40.00	40.00	40.00
Sunflower oil	0.00	20.00	20.00	20.00	20.00	20.00
Wheat bran	38.60	39.50	39.50	39.50	39.50	39.50
Wheat pollard	75.30	75.30	75.30	75.30	75.30	75.30
Fish meal	39.60	27.90	27.90	27.90	27.90	27.90
L-Lysine	2.00	2.00	2.00	2.00	2.00	2.00
DL-Methionine	2.00	2.00	2.00	2.00	2.00	2.00
Di-calcium phosphate	15.00	17.90	17.90	17.90	17.90	17.90
Calcium carbonate	5.00	4.60	4.60	4.60	4.60	4.60
Choline chloride	0.800	0.80	0.80	0.80	0.80	0.80
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[1]	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin premix[2]	1.50	1.50	1.50	1.50	1.50	1.50
Antioxidant, butylated hydroxyanisole	2.00	2.00	2.00	2.00	2.00	2.00
Toxin binder	1.30	1.30	1.30	1.30	1.30	1.30
Arginine	0.00	2.50	2.50	2.50	2.50	2.50
Vitamin E (mg/kg)	-	-	20.00	50.00	100.00	150.00

[1] retinol: 2 mg, α-tocopherol: 0.02 mg, cholecalciferol: 0.03 mg, menadione: 1.33 mg, thiamine: 0.83 mg, cobalamin: 0.03 mg, riboflavin: 2.0 mg, biotin: 0.03 mg, folic acid: 0.33 mg, niacin: 23.30 mg, pantothenic acid: 3.75 mg, and pyridoxine: 1.33 mg
[2] iron: 120 mg, zinc: 100 mg, manganese: 150 mg, copper: 20 mg, magnesium: 12 mg, cobalt: 0.6 mg, selenium: 0.20 mg
T1: 6% palm oil, T2: blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E

Table 2 Nutritional composition of experimental starter diets for broilers

Nutrient content	Dietary treatment					
	T1	T2	T3	T4	T5	T6
Metabolizable energy, MJ/kg	12.86	12.86	12.86	12.86	12.86	12.86
Crude protein, g/kg	210.10	210.10	210.10	210.10	210.10	210.10
Fat g/kg	82.50	82.00	82.00	82.00	82.00	82.00
Fibre, g/kg	42.90	43.60	43.60	43.60	43.60	43.60
Calcium, %	10.20	10.20	10.20	10.20	10.20	10.20
Available P, g/kg	4.60	4.60	4.60	4.60	4.60	4.60
Lysine, %	13.33	13.33	13.33	13.33	13.33	13.33
Methionine, %	5.50	5.40	5.40	5.40	5.40	5.40
Arginine, g/kg	13.80	16.30	16.30	16.30	16.30	16.30
Meth+ cyst, g/kg	8.80	8.70	8.70	8.70	8.70	8.70
Tryptophan, g/kg	2.60	2.60	2.60	2.60	2.60	2.60
α-tocopherol, mg/kg	40.72	37.83	50.68	78.45	124.49	165.73

T1: 6% palm oil, T2: blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E

At 42 days old, 12 birds per treatment (two replicates) were randomly selected and slaughtered to sample the breast muscle. These samples were cut into five portions (about 80 to 100 g) and each portion was allocated to one of five ageing periods, that is, 0, 1, 5, 7, and 10 days post-mortem. The day 0 samples were snap frozen in liquid nitrogen immediately after collection and stored at -80 °C. The samples for the subsequent ageing periods were vacuum-packed and refrigerated (4 °C). After completion of the ageing periods, the muscle samples were taken from the refrigerator and cut into sub samples. Then, the sub samples were kept at -20 °C to analyse meat quality or at -80 °C to determine their fatty acid profile, lipid oxidation, vitamin E, thiol and carbonyl contents.

The fatty acids were extracted from breast muscle samples (1g) using a mixture of chloroform and methanol at a ratio of 2: 1 (v/v) (Folch *et al.*, 1957, in Khatun *et al.*, 2017). Determination of α-tocopherol from the meat samples followed the method of Rutkowski and Grzegorczyk (2007) using an appropriate standard, a blank sample, and the meat samples. The absorbance values of the test samples and standard were measured against the blank at 539 nm wavelength using a spectrophotometer. The α-tocopherol content was calculated with this formula:

$$\text{α-tocopherol content (μM)} = (A_x/A_s) \times C_s$$

where: C_s = concentration of standard,
A_x = absorbance of test sample, and
A_s = absorbance of standard.

The pH of breast meat at the post-mortem ageing periods was determined with a portable pH meter (Mettler Toledo, AG 8603, Switzerland). The pH reading was measured in triplicate for each sample and an average of three readings was used to calculate each sample. Drip loss and cooking loss (CL) were measured by calculating the percentage of weight loss, relative to the initial weight (Honikel, 1998) (see also Khatun *et al.*, 2018) using this equation:

DL or CL (%) = [initial weight - final weight) ÷ initial weight] × 100

Table 3 Ingredients of experimental finisher diets for broilers

Ingredient, g/kg	Dietary treatment					
	T1	T2	T3	T4	T5	T6
Corn	495.80	490.90	490.90	490.90	490.90	490.90
Soybean meal	291.60	287.40	287.40	287.40	287.40	287.40
Palm oil	60.00	40.00	40.00	40.00	40.00	40.00
Sunflower oil	0.00	20.00	20.00	20.00	20.00	20.00
Wheat bran	34.00	45.50	45.50	45.50	45.50	45.50
Wheat pollard	50.50	43.40	43.40	43.40	43.40	43.40
Fish meal	27.70	30.40	30.40	30.40	30.40	30.40
L-Lysine	2.00	2.00	2.00	2.00	2.00	2.00
DL-Methionine	2.00	2.00	2.00	2.00	2.00	2.00
Di-calcium phosphate	18.20	18.30	18.30	18.30	18.30	18.30
Calcium carbonate	9.80	9.30	9.30	9.30	9.30	9.30
Choline chloride	0.80	0.80	0.80	0.80	0.80	0.80
Salt	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix[1]	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin premix[2]	1.50	1.50	1.50	1.50	1.50	1.50
Antioxidant, butylated hydroxyanisole	2.00	2.00	2.00	2.00	2.00	2.00
Toxin binder	1.30	1.30	1.30	1.30	1.30	1.30
Arginine	0.00	2.50	2.50	2.50	2.50	2.50
Vitamin E (mg/kg)	-	-	20.00	50.00	100.00	150.00

[1] retinol: 2 mg, α-tocopherol: 0.02 mg, cholecalciferol: 0.03 mg, menadione: 1.33 mg, thiamine: 0.83 mg, cobalamin: 0.03 mg, riboflavin: 2.0 mg, biotin: 0.03 mg, folic acid: 0.33 mg, niacin: 23.30 mg, pantothenic acid: 3.75 mg, and pyridoxine: 1.33 mg
[2] iron: 120 mg, zinc: 100 mg, manganese: 150 mg, copper: 20 mg, magnesium: 12 mg, cobalt: 0.6 mg, selenium: 0.20 mg
T1: 6% palm oil, T2: blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E

Meat tenderness was measured with the sample that had been used to determine CL. Each sample was kept vacuum-packed in a sealed bag to avoid evaporation and stored overnight at 4 °C. The following day, each sample was cut into at least three sub samples (cuboid) 1 cm × 1 cm × 2 cm with the long axis aligned with the muscle fibre direction. Each sub sample was sheared perpendicular to the muscle fibres by the TA.HDplus® texture analyser with a Volodkevitch blade set (Texture Technologies Inc., Hamilton, Massachusetts, USA) (Cavitt *et al.* 2004). Warner-Bratzler shear force (WBSF) values were reported as the average of all sub sample values, as kg unit. Meat samples were taken from the -80 °C freezer and thawed overnight at 4 °C. The colour measurements were conducted using a colour flex spectrophotometer (Hunter Lab Reston, VA, USA) according to AMSA (2012).

Lipid oxidation in muscle samples was measured with the TBARS method (Lynch & Frei, 1993). The malondialdehyde (MDA) value was calculated from a standard curve of 1, 1, 3, 3-tetraethoxypropane, and expressed as mg MDA/kg meat.

Myofibril proteins were extracted according to Morzel *et al.* (2006) with minor modifications (Sabow *et al.*, 2016). Protein concentration was assessed according to Bradford (1976). The standard solutions for protein quantification were prepared with bovine serum albumin. The protein concentration of each sample was determined according to a standard curve of protein concentration against absorbance at 595 nm wavelength. The free thiol concentration was estimated based on Ellman's method using 2, 2-dithiobis (5-nitropyridine) (DTNP) (Winterbourn, 1990) with slight modifications (Morzel *et al.*, 2006). The stock solution containing 4 mg of myofibrillar proteins was dissolved mixed with 3 ml of 100 mM phosphate buffer at pH 8 containing 8 M urea. About 30 µl of 10 mM DTNP was added, followed by incubation for one hour at room

temperature. Absorbance at 386 nm was measured with a spectrophotometer (Spectronic Instruments, ThermoFisher Scientific Inc., Waltham, Massachusetts, USA) against a blank buffer without protein. The absorbance value of the blank was subtracted, and thiol concentration was estimated using an absorption coefficient of 14,000 M–1 cm–1. The results were expressed as nmol of free thiol per mg of protein.

Table 4 Nutritional composition of experimental finisher diets for broilers

Nutrient content	Dietary treatment					
	T1	T2	T3	T4	T5	T6
Metabolizable energy, MJ/kg	13.01	13.01	13.01	13.01	13.01	13.01
Crude protein, g/kg	195.10	195.10	195.10	195.10	195.10	195.10
Fat g/kg	83.10	84.0	84.0	84.0	84.0	84.0
Fibre, g/kg	40.40	40.40	40.40	40.40	40.40	40.40
Calcium, %	12.20	12.20	12.20	12.20	12.20	12.20
Available P, g/kg	4.60	4.60	4.60	4.60	4.60	4.60
Lysine, %	12.30	12.30	12.30	12.30	12.30	12.30
Methionine, %	5.30	5.30	5.30	5.30	5.30	5.30
Arginine, g/kg	12.80	12.80	12.80	12.80	12.80	12.80
Meth+ cyst, g/kg	8.30	8.30	8.30	8.30	8.30	8.30
Tryptophan, g/kg	2.40	2.40	2.40	2.40	2.40	2.40
α-tocopherol, mg/kg	39.38	36.64	47.53	77.37	121.91	164.08

T1: 6% palm oil, T2: a blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E

Carbonyl content was determined according to Oliver *et al* (1987) with slight modification (Botsoglou *et al.*, 2012). Carbonyl concentration was calculated from the absorption of supernatant from the sample and the control tubes at 370 nm. The results were expressed as nmol of carbonyl per mg of protein based on an absorption coefficient of 21.0 mM−1 cm−1.

The data for fatty acid profile were analysed statistically using PROC GLM of SAS (SAS Institute Inc., Cary North Carolina, USA). The differences in the meat quality parameters because of the post-mortem ageing period were determined through repeated measure analysis again using the GLM procedure. The means were separated using the Duncan's multiple range test. A *P*-value of less than 0.05 was considered significant.

Results and Discussion

Bodyweight and bodyweight gain were greater in T2-T6 than in T1. Moreover, FCR was greater *(P* <0.05) in chickens fed T1 than those fed the other treatments. However, there were no significant differences *(P* >0.05) in bodyweight gain and FCR between the chickens fed the various levels of vitamin E and those fed the positive control diet. Feed intake was not affected by the treatments over the rearing periods. The performances of the broiler chickens fed the dietary treatments are shown in Table 5.

The breast meat of T2--T6 had a lower *(P* <0.05) percentage of palmitic and total SFA and a greater *(P* <0.05) percentage of linoleic acid, total UFA, total PUFA, UFA to SFA ratio, and PUFA to SFA ratio compared with T1. The addition of various levels of α-tocopherol had no effect on muscle fatty acid composition except for eicosapentaenoic acid. The effects of oil blend, L-Arginine, and α-tocopherol acetate supplementation on the fatty acid composition of the breast muscle in broiler chickens are presented in Table 6.

Table 5 Growth performance of broilers fed diets differing in oil and vitamin E contents

	Treatment						SE
	T1	T2	T3	T4	T5	T6	
Bodyweight, g	2316.18[b]	2577.23[a]	2542.50[a]	2580.93[a]	2550.65[a]	2530.53[a]	3.53
Bodyweight gain, g	2275.00[b]	2535.86[a]	2500.89 [a]	2539.99[a]	2510.26[a]	2490.20[a]	21.21
Feed Intake, g	3906.38	3995.91	3955.04	3932.80	4003.23	4003.76	18.48
FCR	1.72[a]	1.57[b]	1.58[b]	1.55[b]	1.60[b]	1.60[b]	0.013

[a,b,c] Within a row, means with a common superscript were not different at P =0.05
T1: 6% palm oil, T2: a blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E; FCR: feed conversion ratio

Table 6 Effect of vitamin E supplementation on the meat fatty acid profile of broilers fed diets containing oil blend and L-Arginine

Fatty acid (%)	Treatments						SE
	T1	T2	T3	T4	T5	T6	
Meristic C14:0)	0.77	0.76	0.71	0.74	74	0.67	0.04
Palmitic C16:0)	27.88[a]	24.39[b]	23.85[b]	23.47[b]	23.17[b]	22.39[b]	0.05
Palmitoleic C16:1)	3.30[b]	5.25[a]	5.16[a]	5.27[a]	5.29[a]	4.86[a]	0.21
Stearic C18:0	5.95	4.47	4.58	4.90	4.93	4.81	.015
Oleic C18:1n-9	42.44	39.80	40.58	40.14	39.32	39.67	0.60
Linoleic C18:2n-6	17.24[b]	21.98[a]	21.88[a]	22.05[a]	22.88[a]	23.87[a]	0.56
Linolenic C18:3n-3	0.62[b]	0.90[a]	0.91[a]	0.91[a]	0.93[a]	0.93[a]	0.04
Arachidonic C20:4n-6	0.37[b]	0.71[a]	0.71[a]	0.72[a]	0.74[a]	0.79[a]	0.03
Eicosapentaenoic C20:5n-3	0.68[d]	0.85[bcd]	0.81[dc]	0.93[bc]	1.03[ab]	1.21[a]	0.05
Docosapentaenoic C22:5n-3	0.43	0.45	0.44	0.44	0.44	0.43	0.03
Docosahexaenoic C22:6n-3	0.34	0.41	0.40	0.43	0.44	0.45	0.02
SFA[1]	34.60[a]	29.61[b]	29.14[b]	29.11[b]	28.85[b]	27.87[b]	0.50
UFA[2]	65.40[b]	70.39[a]	70.86[a]	70.89[a]	71.15[a]	72.13[a]	0.50
MUFA[3]	45.74	45.06	45.74	45.41	44.62	44.54	0.52
PUFA[4]	19.67[b]	25.33[a]	25.12[a]	25.48[a]	26.54[a]	27.60[a]	0.6
PUFA n-6[5]	17.91[b]	22.83[a]	22.62[a]	22.98[a]	24.02[a]	25.08[a]	0.58
PUFA n-3[6]	1.75[b]	2.50[a]	2.50[a]	2.51[a]	2.52[a]	2.52[a]	0.09
n-6: n-3	10.28	9.71	9.08	9.30	10.18	10.16	0.30
UFA: SFA	1.89[b]	2.39[a]	2.44[a]	2.44[a]	2.47[a]	2.61[a]	0.05
PUFA: SFA	0.57	0.86	0.86	0.88	0.97	0.99	0.03

[a,b,c] Within a row, means with a common superscript were not different at *P* =0.05
T1: 6% palm oil, T2: a blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E
[1]SFA: saturated fatty acids = C14:0 + C16:0 + C18:0, UFA: unsaturated fatty acids = C16:1 + C18:1n-9 + C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3+ C22:5n-3; MUFA: monosaturated fatty acids = C16:1 + C18:1n-9, PUFA: polyunsaturated fatty acids = C18:2n-6 + C18:3n-3 + C20:4n-6 + C20:5n-3 + C22:5n-3, PUFA n-6: C18:2n-6+ C18:3n-6+ C20:4n-6. PUFA n-3: C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3

Vitamin E supplementation significantly affected the α-tocopherol content of the breast muscle at various periods of post-mortem ageing in broiler chickens fed diets differing in oil blend and L-Arginine The α-tocopherol concentration in the meat rose (*P* <0.05) with increasing levels of vitamin E in the diet,

particularly with the inclusion of 50 mg/kg vitamin E or more in the diet (T4, T5 and T6). The α-tocopherol concentration decreased with the increased duration of post-mortem ageing.

On days 5 and 7 post mortem, the treatments influenced $(P <0.05)$ the DL of breast muscle in broiler chickens. Drip loss in T4, T5 and T6 was lower $(P <0.05)$ than in the other treatments. Irrespective of treatment, DL increased with post-mortem ageing, whereas no further changes of DL were observed from five to seven post-mortem storage days for T4 to T6. No significant differences $(P >0.05)$ were observed in the CL of breast muscle among the treatments and post-mortem ageing periods. The DL, CL, pH, WBSF values, and colour coordinates of breast muscle of broiler chickens fed various treatments are presented in Table 7.

Supplementation of vitamin E in the broiler diet increased the tenderness of meat. On day 1 post-mortem, the WBSF values in T4, T5 and T6 diets were lower $(P <0.05)$ than T1 and T2 diets. The WBSF value in T3 birds was intermediate did not differ $(P >0.05)$ from either T1 and T2 or T4, T5 and T6. On day 5 post-mortem, WBSF values in birds fed the T4, T5, and T6 diets were lower $(P <0.05)$ than the other treatments. On day 7 post-mortem, treatments had no effect $(P >0.05)$ on WBSF values. Regardless of treatment, WBSF decreased significantly with increased post-mortem ageing. The WBSF value of breast muscle on days 5 and 7 did not differ $(P >0.05)$ in T4, T5 and T6.

Treatments had no effect $(P >0.05)$ on the pH values of the breast muscle at any post-mortem ageing period. The meat pH values on day 0 post-mortem were higher than day 1 post mortem across treatments. No changes were observed in the meat pH values on days 1, 5, and 7 post-mortem in the birds fed the T4, T5 and T6 diets.

Dietary treatment had no effect $(P >0.05)$ on the lightness (L*) and yellowness (b*) values of the breast muscle at any post-mortem ageing period. On days 1 and 5 post-mortem, the a* value rose $(P <0.05)$ as the level of vitamin E increased in diet. The a* values decreased $(P <0.05)$ as post-mortem ageing increased.

Thiobarbituric acid-reactive substance values rose $(P <0.05)$ across all treatments as the post-mortem ageing period increased. Vitamin E $(P <0.05)$ influenced lipid peroxidation (TBARS value) in all the post-mortem ageing periods except on days 0 and 1 post-mortem. At 5, 7, and 10 days post-mortem, TBARS values were lower in the chicken fed the T4, T5, and T6 diets than the other treatments.

On days 0, 1 and 5 post-mortem, treatment had no effect on the meat free thiol content. On days 7 and 10 post-mortem, the free thiol content in T4, T5, and T6 was greater $(P <0.05)$ compared with the other treatments. Irrespective of treatment, thiol content reduced $(P <0.05)$ with post-mortem ageing. Dietary treatment had no effect on the meat carbonyl content at any post-mortem ageing period. Regardless of treatment, carbonyl content increased with post-mortem ageing. The effects of treatments and post-mortem ageing period on lipid oxidation, free thiol content and carbonyl content in breast muscle of broiler chickens are shown in Table 8.

The increased bodyweight and bodyweight gain in T2--T6 could be attributed to the greater degree of unsaturation of the oil blend compared with the single oil in the control diet. Moreover, L-Arginine is a substrate that is involved in biosynthesis of protein, polyamines, glutamine, proline, and ornithine which are crucial to animal growth (Khajali & Wideman, 2010). Chickens fed the oil blend and L-Arginine increased bodyweight significantly compared with the control diet. These results were consistent with those of Emadi *et al.* (2011) and Wu *et al.* (2011), who reported that L-Arginine increased the growth performance of chickens and ducks, respectively

The fatty acid composition of chicken muscle is attributed to the synthesis of de novo fatty acid from protein and carbohydrate precursors and fatty acids. In the current study, supplementation of the oil blend and L-Arginine reduced the concentrations of Palmitic acid and total SFA, and increased the concentrations of palmitoleic acid, linoleic acid, eicosapentaenoic acid, and total UFA in breast muscle. These results were consistent with those of Velasco *et al.* (2010) and Ma *et al.* (2015), who observed a similar pattern of fatty acid profile changes in the meat of chicken and pigs, respectively. However, vitamin E supplementation did not affect the meat fatty acid profile in the current study. This observation is consistent with that of Zduńczyk *et al.* (2011), who reported that meat fatty acid compositions were altered by fat type, but not by vitamin E, when diet was supplemented with vitamin E and various oil sources.

Table 7 Effect of vitamin E supplementation on meat vitamin E content and meat quality at various post-mortem ageing period of broilers fed diets containing oil blend and L-Arginine

	Days post-mortem	Treatment					
		T1	T2	T3	T4	T5	T6
Vitamin E (α-tocopherol) (mg/kg)	1	$5.68^{dcx} \pm 0.09$	$5.14^{dx} \pm 0.08$	$6.37^{cx} \pm 0.10$	$10.10^{bx} \pm 0.11$	$13.06^{ax} \pm 0.09$	$13.68^{ax} \pm 0.11$
	5	$4.91^{dy} \pm 0.08$	$4.36^{ey} \pm 0.09$	$5.26^{cy} \pm 0.06$	$8.36^{by} \pm 0.06$	$11.87^{ay} \pm 0.07$	$12.08^{ay} \pm 0.09$
	7	$2.98^{dz} \pm 0.08$	$2.59^{ez} \pm 0.06$	$3.33^{dz} \pm 0.06$	$6.09^{cz} \pm 0.06$	$7.42^{bz} \pm 0.08$	$7.79^{az} \pm 0.07$
Drip loss, %	1	$1.87^{z} \pm 0.21$	$1.88^{z} \pm 0.29$	$1.86^{z} \pm 0.37$	$1.22^{y} \pm 0.41$	$1.26^{y} \pm 0.38$	$1.05^{y} \pm 0.20$
	5	$4.37^{ay} \pm 0.29$	$4.39^{ay} \pm 0.38$	$4.37^{ay} \pm 0.31$	$3.56^{abx} \pm 0.36$	$3.27^{bx} \pm 0.33$	$3.26^{bx} \pm 0.27$
	7	$5.77^{ax} \pm 0.59$	$5.81^{ax} \pm 0.57$	$5.48^{ax} \pm 0.38$	$4.36^{bx} \pm 0.40$	$4.23^{bx} \pm 0.31$	$4.16^{bx} \pm 0.25$
Cooking loss, %	1	22.72 ± 0.69	23.26 ± 0.79	20.65 ± 0.96	20.11 ± 0.75	21.49 ± 0.61	21.93 ± 0.84
	5	23.21 ± 0.20	23.58 ± 0.34	$22.02 \pm 0..37$	21.70 ± 0.60	22.67 ± 0.66	22.30 ± 0.63
	7	21.48 ± 0.74	21.85 ± 0.62	19.89 ± 0.95	19.53 ± 0.47	20.37 ± 0.92	20.74 ± 0.68
Shear force, kg	1	$1.27^{ax} \pm 0.04$	$1.26^{ax} \pm 0.03$	$1.20^{xab} \pm 0.04$	$1.14^{bx} \pm 0.04$	$1.14^{bx} \pm 0.03$	$1.15^{bx} \pm 0.04$
	5	$1.14^{ay} \pm 0.04$	$1.13^{ay} \pm 0.04$	$1.10^{ay} \pm 0.03$	$1.01^{by} \pm 0.02$	$1.01^{by} \pm 0.01$	$1.02^{by} \pm 0.02$
	7	$1.02^{z} \pm 0.03$	$1.00^{z} \pm 0.03$	$0.98^{z} \pm 0.03$	$0.98^{y} \pm 0.02$	$0.99^{y} \pm 0.01$	$0.98^{y} \pm 0.02$
pH of meat	0	$6.30^{x} \pm 0.040$	$6.27^{x} \pm 0.04$	$6.29^{x} \pm 0.04$	$6.35^{x} \pm 0.02$	$6.37^{x} \pm 0.03$	$6.37^{x} \pm 0.03$
	1	$5.89^{y} \pm 0.06$	$5.88^{y} \pm 0.02$	$5.88^{y} \pm 0.01$	$5.96^{y} \pm 0.06$	$5.97^{y} \pm 0.05$	$5.95^{y} \pm 0.06$
	5	$5.91^{y} \pm 0.03$	$5.94^{y} \pm 0.04$	$5.94^{y} \pm 0.03$	$5.97^{y} \pm 0.03$	$5.99^{y} \pm 0.08$	$5.99^{y} \pm 0.05$
	7	$6.23^{x} \pm 0.01$	$6.21^{x} \pm 0.01$	$6.19^{x} \pm 0.02$	$6.08^{y} \pm 0.02$	$6.09^{y} \pm 0.08$	$6.09^{y} \pm 0.05$
L* (lightness)	1	51.21 ± 0.73	50.14 ± 1.89	48.68 ± 1.30	48.29 ± 2.41	48.21 ± 0.89	47.75 ± 1.11
	5	50.23 ± 0.66	50.72 ± 0.70	51.11 ± 1.00	49.54 ± 2.04	49.75 ± 0.71	49.61 ± 1.58
	7	51.68 ± 0.82	51.23 ± 0.86	50.21 ± 0.90	50.23 ± 1.70	50.35 ± 0.86	51.87 ± 0.99
a* (redness)	1	$5.28^{bx} \pm 0.20$	$5.48^{bx} \pm 0.23$	$6.06^{bx} \pm 0.24$	$7.33^{ax} \pm 0.79$	$7.81^{ax} \pm 0.46$	$7.79^{ax} \pm 0.30$
	5	$4.92b^{xy} \pm 0.27$	$5.11^{bx} \pm 0.29$	$5.13^{bxy} \pm 0.48$	$6.46^{axy} \pm 0.24$	$6.71^{ax} \pm 0.44$	$6.86^{ax} \pm 0.49$
	7	$4.16^{y} \pm 0.35$	$4.22^{y} \pm 0.34$	$4.34^{y} \pm 0.26$	$4.70^{y} \pm 0.44$	$5.23^{y} \pm 0.44$	$4.98^{y} \pm 0.41$
b* (yellowness)	1	13.96 ± 0.56	14.27 ± 0.26	14.43 ± 0.34	14.69 ± 0.49	15.14 ± 0.58	15.12 ± 0.45
	5	13.61 ± 0.23	13.85 ± 0.33	13.78 ± 0.47	14.01 ± 0.47	14.28 ± 0.41	14.30 ± 0.46
	7	13.31 ± 0.52	13.62 ± 0.41	13.71 ± 0.32	13.22 ± 0.34	13.84 ± 0.44	13.94 ± 0.43

[x,y,z] Within a column, means with a common superscript were not different at $P =0.05$
[a,b,c,d] Within a row, means with a common superscript were not different at $P =0.05$
T1: 6% palm oil, T2: a blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E.

Table 8 Effects of dietary treatment and post-mortem ageing period on thiobarbituric acid-reactive substances (mg malondialdehyde/kg), free thiol and carbonyl contents in meat of broiler chickens.

	Days post-mortem	Treatments					
		T1	T2	T3	T4	T5	T6
TBARS mg MDA/ kg	0	$0.27^z \pm 0.02$	$0.29^z \pm 0.02$	$0.26^z \pm 0.02$	$0.23^z \pm 0.01$	$0.24^z \pm 0.02$	$0.22^z \pm 0.01$
	1	$0.42^z \pm 0.03$	$0.49^y \pm 0.03$	$0.41^{yz} \pm 0.02$	$0.36^y \pm 0.05$	$0.36^z \pm 0.04$	$0.33^z \pm 0.04$
	5	$0.63^{by} \pm 0.04$	$0.75^{ax} \pm 0.02$	$0.61^{by} \pm 0.02$	$0.50^{cx} \pm 0.02$	$0.47^{cy} \pm 0.02$	$0.44^{cy} \pm 0.02$
	7	$1.64^{bx} \pm 0.03$	$1.79^{aw} \pm 0.02$	$1.69^{abx} \pm 0.04$	$1.19^{cw} \pm 0.02$	$1.18^{cx} \pm 0.02$	$1.16^{cx} \pm 0.04$
	10	$3.27^{aw} \pm 0.18$	$3.69^{av} \pm 0.09$	$3.35^{aw} \pm 0.14$	$1.48^{bv} \pm 0.14$	$1.46^{bw} \pm 0.15$	$1.40^{bw} \pm 0.12$
Thiol (nmol/mg protein)	0	$52.00^w \pm 0.56$	$51.6^w \pm 0.72$	$52.11^w \pm 0.49$	$53.00^w \pm 0.59$	$53.56^w \pm 0.58$	$53.06^w \pm 0.58$
	1	$51.00^w \pm 0.78$	$50.57^w \pm 0.79$	$50.64^w \pm 0.45$	$52.38^w \pm 0.37$	$52.19^w \pm 0.40$	$51.90^w \pm 0.40$
	5	$46.17^x \pm 0.23$	$45.54^x \pm 0.23$	$46.35^x \pm 0.27$	$47.47^x \pm 0.63$	$47.73^x \pm 0.58$	$47.40^x \pm 0.56$
	7	$41.14^{bcy} \pm 0.67$	$39.47^{cy} \pm 0.45$	$42.60^{by} \pm 0.69$	$45.36^{ay} \pm 0.49$	$45.59^{ay} \pm 0.47$	$45.04^{ay} \pm 0.47$
	10	$36.01^{bz} \pm 0.72$	$35.24^{bz} \pm 0.47$	$36.43^{bz} \pm 0.65$	$40.15^{az} \pm 0.72$	$41.42^{az} \pm 0.58$	$41.97^{az} \pm 0.59$
Carbonyl (nmol/mg protein)	0	$1.35^w \pm 0.03$	$1.36^v \pm 0.01$	$1.34^w \pm 0.02$	$1.31^v \pm 0.01$	$1.29^v \pm 0.01$	$1.29^v \pm 0.02$
	1	$1.43^w \pm 0.02$	$1.44^w \pm 0.01$	$1.41^w \pm 0.02$	$1.40^w \pm 0.01$	$1.39^w \pm 0.01$	$1.39^w \pm 0.01$
	5	$2.30^x \pm 0.02$	$2.30^x \pm 0.02$	$2.33^x \pm 0.01$	$2.31^x \pm 0.01$	$2.30^x \pm 0.01$	$2.30^x \pm 0.01$
	7	$3.07^y \pm 0.06$	$3.10^y \pm 0.02$	$3.05^y \pm 0.04$	$2.98^y \pm 0.03$	$2.95^y \pm 0.06$	$3.00^y \pm 0.05$
	10	$4.59^z \pm 0.05$	$4.63^z \pm 0.04$	$4.57^z \pm 0.02$	$4.53^z \pm 0.01$	$4.52^z \pm 0.02$	$4.53^z \pm 0.01$

[v,w,x,y,z] Within a column, means with a common superscript were not different at $P = 0.05$
[a,b,c] Within a row, means with a common superscript were not different at $P = 0.05$
T1: 6% palm oil, T2: a blend of 4% palm oil and 2% sunflower oil + 0.25% L-Arginine, T3: T2+ 20 mg/kg vitamin E, T4: T2 + 50 mg/kg vitamin E, T5: T2 + 100 mg/kg vitamin E and T6: T2 + 150 mg/kg vitamin E; TBARS: thiobarbituric acid-reactive substances; MDA: malondialdehyde

In the current study, meat α-tocopherol concentration increased with the rising level of supplementation in the broiler diet. Skřivan *et al.* (2010) also observed greater levels of α-tocopherol in the breast and thigh muscle with increased levels of α-tocopherol in a broiler diet. Giraudeau *et al.* (2013), suggested that the increased α-tocopherol concentration in meat could protect the cell membrane from oxidative damage caused by unsaturated phospholipids. However, as post-mortem ageing continued, the α-tocopherol concentration decreased, possibly because of the interruption of the antioxidant defence system and augmented lipid oxidation with increased post-mortem storage time. Adeyemi *et al.* (2015) similarly reported decreased vitamin E content with time in post-mortem-aged chevon. Water-holding capacity (WHC) of meat is a vital index for assessing meat quality. Vitamin E is soluble in fat and plays a vital role in upholding the integrity of the cell membrane (Khan *et al.*, 2011), which can improve WHC and reduce fluid loss from the muscle. In the current study, vitamin E supplementation reduced DL in the breast muscle of broiler chickens. Li *et al.* (2009) also found that vitamin E supplementation (100 to 200 mg/kg) reduced DL in the thigh and breast muscles. As expected, DL increased over the post-mortem ageing period in the current study. This could be attributed to the disruption of collagen and myofibrillar proteins during ageing, which causes the myofibrillar proteins to lose their WHC (Abdulla *et al.*, 2017). Karami *et al.* (2011) also observed increased DL over the post-mortem ageing period in chevon. The lack of significant trend over time in DL from the breast muscle of broiler chickens fed the diets supplemented with vitamin E might be the result of a protective effect of vitamin E on the cell membrane.

A combination of fluid and soluble substances is lost from the meat during cooking (Lawrie & Ledward, 2006). Like Zhang *et al.* (2011), vitamin E had no effect on CL in the current study. Irrespective of treatment, post-mortem aging time had no effect on CL. This could be because of the similar meat pH values that were observed over the post-mortem ageing period since pH has previously been shown to be associated with CL (Dalle Zotte *et al.*, 1995). The current findings were consistent with those of Zhang *et al.* (2013b), who reported no significant differences in the ultimate pH value of broiler breast meat following supplementation with various levels of vitamin E.

Dietary supplementation of vitamin E influenced the WBSF values of broiler breast meat. Zhang *et al.* (2013b) also found that vitamin E decreased meat WBSF and increased WHC. The WBSF decreased with post-mortem ageing in the current study as expected, perhaps because of the weakening of myofibrillar structure by endogenous muscle proteinases during ageing. This finding was in tandem with those results reported by Abdulla *et al.* (2017), who found reduced WBSF values of broiler breast meat as the post-mortem ageing period increased.

Breast meat pH was not affected by vitamin E supplementation at any post-mortem ageing period. This could be because of similar management practices and slaughter processes during the experiment. Irrespective of treatment, breast meat pH declined at day 1 post mortem compared with day 0 post mortem. This decrease might be because of anaerobic glycolysis in the post-mortem muscle, which is required to convert muscle glycogen to lactic acid. A pH value of chicken meat lower than 5.7 at day 1 post mortem is considered poor quality because of low WHC (Corzo *et al.*, 2009). In the present study, the meat pH values on day 1 post-mortem for all treatments were within the normal range (Abdulla *et al.*, 2015). Across all treatments the pH values of the meat differed according to then number of days post mortem. This suggests a stability of muscle oxidative status induced by vitamin E. However, the pH value of meat from chickens that were not supplemented with vitamin E (T1, T2) or had a low level of vitamin E (T3) increased at day 7 post mortem. This could be because of increased meat alkalization caused by spoilage organisms (Abdulla *et al.*, 2015).

During slaughter, the antioxidant level of muscle plays an important part in determining colour stability (Ponnampalam *et al.*, 2012). In the present study, increased vitamin E in the diets enhanced a* values and conserved the colour of meat by reducing lipid oxidation, which indirectly delayed myoglobin oxidation. Similar findings were reported by Zhang *et al.* (2013b), Li *et al.* (2003), and Santé-Lhoutellier *et al.* (2008).

Lipid oxidation affects the colour, flavour and nutritional value of meat. The high meat TBARS values observed for the birds fed T2 could be attributed to the lack of antioxidant and relatively higher amount of PUFA in meat from this group. However, significant reductions in TBARS values were observed in T4, T5 and T6 compared with T1 and T2. This might be attributed to the increased content of α-tocopherol in the meat of birds supplemented with vitamin E compared with the other groups. These findings are consistent with those of Giraudeau *et al.* (2013) and Coetzee and Hoffman (2001).

A TBARS value greater than 1.5 mg/kg meat is associated with an objectionable aroma owing to lipid oxidation and it is not suitable for further storage (Buckley & Morrissey, 1992; Coetzee & Hoffman, 2001), In the present study, TBARS values on days 0, 5, 7 and 10 post mortem were consistently less than 1.5 mg/kg in the meat from broilers fed T3 – T6. The implication of the current observation was that supplementation of at least 50 mg/kg vitamin E with oil blend and L-Arginine improved the shelf life of meat between 7 and 10 days of refrigeration (4 °C). On the other hand, the meat from the non-supplemented group should not be

stored more than five days at 4 °C. These observations corroborate the findings of Voljc *et al.* (2011) that vitamin E supplementation can markedly decrease lipid oxidation and extend meat shelf life. Nonetheless, a detailed microbiological analysis is needed to substantiate this assertion.

In the current study, the increased meat thiol content observed in T4 - T6 on days 7 and 10 post mortem was in line with the findings of Batifoulier *et al.* (2002), who showed that supplementation of vitamin E with 6% soybean oil protected the free thiol groups from oxidation in turkey muscle compared with those fed the control diet. In the present study, the free thiol concentration in meat decreased over the post-mortem ageing period consistent with results from Sabow *et al.* (2015) in a study of chevon. This could be because of the oxidation of the amino acid side chain (Lund *et al.*, 2011 or a decrease in solubility and other catalytic activities, which enhanced the aggregation and degradation of protein (Nieto *et al.*, 2013).

Carbonyl is a product of oxidized proteins and it is considered a useful indicator of protein oxidation in food (Zhang *et al.*, 2013a). However, carbonyl is only one group of amino acid, which should not be considered representative of the whole protein oxidation phenomena. In the current study, treatments had no effect on the meat carbonyl content. Similarly, dietary supplementation of vitamin E was found to have no effect on carbonyl content in turkey meat (Batifoulier *et al.*, 2002). However, the meat carbonyl content increased over the post-mortem ageing period, a result which is similar to the observed increase in carbonyl content in goat meat that was aged 7 days (Adeyemi *et al.*, 2015).

Conclusion

The diets T2 - T6 produced increased growth and improved the meat quality in broiler chickens. Vitamin E decreased meat DL, increased redness, improved tenderness, and increased the lipid oxidative stability of meat during post-mortem ageing. However, these diets had no effect on the meat fatty acid profile relative to T1. In addition, vitamin E increased the free thiol content of meat on day 7 post mortem. No differences in the meat quality, lipid oxidation and thiol content were attributable to the level of vitamin E supplementation. Thus, supplementation of vitamin E at 50 mg/kg with an oil blend and L-Arginine could be used to improve growth, meat quality and meat shelf life.

Authors' Contributions

JK conducted the experiment and analysed the meat samples; JK, TCL, HA and HLF designed and supervised the research; JK, RM and NS collected data and evaluated the carcasses. JK drafted the manuscript; JK, TCL, HLF, HA and RM reviewed and finalized the writing.

Conflict of Interest Declaration

No potential conflict of interest was reported by the authors.

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