

Effect of vitamin E and omega-3 fatty acids early posthatch supplementation on reducing the severity of wooden breast myopathy in broilers

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ABSTRACT The wooden breast (**WB**) myopathy is identified by the palpation of a rigid pectoralis major (**P. major**) muscle and is characterized as a fibrotic, necrotic P. major muscle disorder in broilers resulting in reduced breast meat quality. Breast muscle affected with WB is under severe oxidative stress and inflammation. The objectives were to identify the effects of dietary vitamin E (**VE**) and omega-3 (**n-3**) fatty acids independently or in combination when fed during the starter phase (0–10 D) or grower phase (11–24 D) on growth performance, meat yield, meat quality, and severity of WB myopathy and to determine the most beneficial dietary supplementation period. A total of 210 Ross 708 broiler chicks were randomly assigned into 7 experimental groups with 10 replicates of 3 birds each. The control group was fed with corn–soybean meal basal diet with VE (10 IU/kg) and n-3 fatty acids (n-6/n-3 ratio of 30:1) at a standard level during the entire study (0–58 D).

Supplementation of VE (200 IU/kg), n-3 fatty acids (n-6/n-3 ratio of 3:1), or combination of both was performed during the starter phase or grower phase. Growth performance, meat yield, meat quality, and WB scores were obtained. There was no significant difference in final body weight and meat yield when VE was increased ($P > 0.05$). In contrast, n-3 fatty acids supplementation in starter diets significantly decreased final body weight, hot carcass weight, and chilled carcass weight of broilers ($P \leq 0.05$). The P. major muscle from broilers supplemented with VE in starter diets had lower shear force than in grower diets ($P \leq 0.05$). Supplemental VE reduced the severity of WB and in starter diets showed a more beneficial effect than those fed VE in the grower diets. These data are suggestive that additional supplementation of dietary VE may reduce the severity of WB and promote breast meat quality without adversely affecting growth performance and meat yield.

Key words: broiler, meat quality, omega-3 fatty acid, vitamin E, wooden breast

2020 Poultry Science 99:2108–2119

<https://doi.org/10.1016/j.psj.2019.12.033>

INTRODUCTION

The poultry industry has increased broiler production by selecting for growth, feed conversion, and muscle mass accretion including pectoralis major muscle (**P. major**) or breast muscle yield. Breast muscles from meat-type commercial broilers are 10 times larger than those from broilers marketed in 1955 at the same age (Collins et al., 2014). As a result of these selection practices, meat quality challenges (Petracci et al., 2013; Mazzoni et al., 2015; Tasoniero et al., 2016) and myopathies (Kuttappan et al., 2012b; Sihvo et al.,

2014; Velleman, 2015) have arisen in the breast muscles of modern commercial broilers. Among the myopathies, wooden breast (**WB**) is of great concern to the poultry industry as it is present within the broiler industry worldwide (Sihvo et al., 2014; Kuttappan et al., 2016; Baldi et al., 2018). This myopathy has created considerable economic losses for the industry because of product downgrades and negative consumer attitudes toward WB breast meat (Sihvo et al., 2014).

Wooden breast is identified by the palpation of a rigid P. major muscle and has moderate or severe myodegeneration along with different levels of myofiber necrosis (Papah et al., 2017; Baldi et al., 2018), fibrosis (Sihvo et al., 2014; Velleman and Clark, 2015), and inflammatory cell accumulation (Sihvo et al., 2014, 2017). Presence of WB often coexists with white striping (**WS**) (Abasht et al., 2016). White striping is characterized by white striation of intramuscular fat and connective tissue parallel to muscle fibers mainly

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Received September 27, 2019.

Accepted December 2, 2019.

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in the P. major muscle (Kuttappan et al., 2012a). The WB myopathies not only lead to histological changes but also to carcass and meat quality reduction. Studies have shown that WB affected tissues have lower water holding capacity (Petracci et al., 2013; Soglia et al., 2016; Sanchez Brambila et al., 2017) and decreased tenderness (Petracci et al., 2015; Tasoniero et al., 2016) compared with normal tissues. Nutritional values of WB affected muscle are significantly changed with higher moisture and lipid content (Petracci et al., 2014; Mazzoni et al., 2015).

Although the exact etiology of WB remains unknown, several studies have indicated that WB affected broilers are under severe oxidative stress (Mutryn et al., 2015; Abasht et al., 2016) and inflammation (Sihvo et al., 2014; Mudalal et al., 2015). Because posthatch muscle growth is dependent on myogenic stem cell called satellite cells, which have their maximal activity during the first week posthatch (Mozdziak et al., 2002) and are sensitive to nutritional changes (Halevy et al., 2000; Powell et al., 2014; Velleman et al., 2014), early posthatch nutritional strategies to reduce oxidative stress and inflammation will likely decrease the incidence and severity of WB myopathies.

Among the numerous antioxidants, vitamin E (VE) is a very powerful lipid-soluble antioxidant that prevents oxidative damage in cells and tissue (Voljč et al., 2011). According to the National Research Council (NRC: National Research Council, 1994), the nutrient requirement of VE for broilers is 10 mg/kg of the diet. However, the latest NRC requirements were revised in 1994. According to the National Chicken Council (2019), the weight of broilers at market age has increased 35% since 1994. In addition, higher levels of oxidative stress as a result of rapid growth may require higher levels of VE. Therefore, the latest NRC guidelines may not reflect the current VE requirement for modern broilers.

Omega-3 (n-3) fatty acids are polyunsaturated fatty acids (PUFA) and have been found to exert anti-inflammatory effects through altering proinflammatory eicosanoids and cytokines profiles (Simopoulos, 2002; Calder, 2003; Rahimi et al., 2011; Yu et al., 2018). Linoleic acid (C18:2) and linolenic acid (C18:3) are dietary essential fatty acids for poultry (Reiser and Gibson, 1950). They are also the precursors for synthesis of very long-chain PUFA, arachidonic acid, and eicosapentanoic acid, which are the precursors of inflammatory eicosanoids. Although the NRC has not given a recommended ratio of omega-6 (n-6)/n-3 in poultry diets, a ratio of 1:1 to 4:1 of n-6/n-3 in diets for humans are suggested to achieve optimal health benefits (Simopoulos, 2002). Commercial poultry diets commonly use diets with n-6/n-3 ratio of over 20:1 and therefore contain very low contents of n-3 fatty acids (Cherian, 2008). The low n-3 fatty acids contents are from a high percentage of corn oil in the diets. As opposed to corn oil, fish oil is a well-known source that can enrich n-3 fatty acids (Bharath et al., 2017).

However, a very high level of fish oil, especially when incorporated in finisher diets, could increase off flavors and oxidation in meat products (Lopez-Ferrer et al., 2001). Furthermore, supplemental VE could be added to obtain better immune function (Talescu et al., 2011) and potentially reduce inflammatory damage associated with the onset of WB.

Although previous studies have found the antioxidant effect of VE (Voljč et al., 2011) and anti-inflammatory effect of n-3 fatty acids to be beneficial (Simopoulos, 2002; Calder, 2003; Rahimi et al., 2011; Yu et al., 2018), there are no published reports using VE and n-3 fatty acids to reduce the WB myopathy or determining the optimal period to administer VE and n-3 fatty acids. Thus, the objective of the present study was to identify the effects of increasing VE, n-3 fatty acids, and combination of both during the starter phase (0–10 D) or grower phase (11–24 D) on growth performance, meat yield, meat quality, and severity of WB myopathy.

MATERIALS AND METHODS

Birds and Experimental Diets

All bird activities were approved by the Institutional Animal Care and Use Committee of The Ohio State University. A total of 210 commercial Ross 708 broiler chicks were individually weighed, wing banded, and placed into pens immediately after hatch. Broilers had *ad libitum* access to feed and water. They were randomly divided into 7 experimental groups with 10 replications of 3 birds each in a completely randomized design. The control group was fed a corn–soybean meal basal diet with VE (Dl- α -tocopherol acetate, 10 IU/kg) and n-3 fatty acids (n-6/n-3 ratio of 30:1) at a standard level during starter phase (0–10 D), grower phase (11–24 D), and finisher phase (25–58 D). Additional supplemental VE or n-3 fatty acids were fed during the starter or grower phase. For the starter dietary supplementation, starter VE, starter n-3, and starter VE and n-3 groups were fed with the basal diet supplemented at concentrations of 200 IU/kg diet of VE, n-3 fatty acids with a n-6/n-3 ratio of 3:1, or a combination of both. The grower and finisher diets were the same as the control group. For the grower dietary supplementation, grower VE, grower n-3, and grower VE and n-3 groups were fed the basal diets supplemented at concentrations of 200 IU/kg diet of VE, n-3 fatty acids with a n-6/n-3 ratio of 3:1, or a combination of both. The starter and finisher diets were the same as the control group. Diets were formulated to meet or exceed all NRC (National Research Council, 1994) nutritional requirements and recommendations in Aviagen's Ross broiler production handbook (Aviagen, 2016). Feed ingredients and calculated nutrient composition in the starter phase are shown in Table 1. Grower diets and finisher diets are shown in Table 2 and Table 3, respectively.

Table 1. Feed ingredients and calculated nutritional composition of starter diets.¹

Item	Control	Starter VE	Starter n-3	Starter VE and n-3
Ingredients, % as-fed				
Corn	50.85	50.83	50.85	50.83
Soybean meal	33.71	33.71	33.71	33.71
Poultry byproduct meal	7.50	7.50	7.50	7.50
Sodium chloride	0.22	0.22	0.22	0.22
Limestone	1.10	1.10	1.10	1.10
Dicalcium phosphate	0.47	0.47	0.47	0.47
Premix ² (Akey #7)	0.35	0.35	0.35	0.35
L-Lys HCl	0.15	0.15	0.15	0.15
DL-Met	0.34	0.34	0.34	0.34
L-Thr	0.11	0.11	0.11	0.11
NaHCO ₃	0.10	0.10	0.10	0.10
Selenium	0.10	0.10	0.10	0.10
Amprolium	1.00	1.00	1.00	1.00
DL- α -tocopherol acetate	0.001	0.020	0.002	0.002
Soy oil	0.11	0.11	1.58	1.58
Corn oil	3.42	3.42	0.13	0.13
Fish oil	-	-	2.29	2.29
Hydrogenated coconut oil	0.47	0.47	-	-
Calculated nutrients and energy				
AME, kcal/kg	3,016	3,015	3,018	3,017
Protein, %	23.73	23.73	23.73	23.73
Calcium, %	0.96	0.96	0.96	0.96
Available phosphorus, %	0.48	0.48	0.48	0.48
Digestible Lys, %	1.28	1.28	1.28	1.28
Digestible Met + Cys, %	0.95	0.95	0.95	0.95

¹Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D). Dietary treatments during the grower phase (11–24 D) received the same diets as the control group during the starter phase.

²The premix provides the following per kg of diet: vitamin A, 1,715,000 IU; vitamin D₃, 6,000 IU; vitamin E, 12,600 IU; vitamin B₁₂, 11 μ g; folic acid, 1.5 mg; biotin, 150 μ g; calcium carbonate, 25 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; and ethoxyquin, 150 mg (Provimi North America, Brookville, OH).

Fatty Acids Determination

The concentrations of long-chain PUFA in feed samples were analyzed using an Agilent Technologies 6890N model gas chromatograph equipped with a 5973N mass spectrometric detector (Agilent Technologies, Wilmington, DE) following the method described by Lima et al. (2017). Feed samples were ground to powder in uniform particle size. Preparation of the fatty acid methyl esters was by the method of direct methylation in Wang et al. (2000). HP-23 capillary column was used for separation. Mass spectrometer was operated in electro-impact mode. Helium was used as carrier gas. Each sample was measured in duplicate. Fatty acid composition of the experimental diets in the starter phase, grower phase, and finisher phase are shown in Tables 4 and 5.

Growth Performance

Animal growth was evaluated by measuring weekly body weights throughout the trial. Feed intake (FI) was calculated by monitoring weekly feed disappearance. Average daily gain (ADG) was calculated as the rate of body weight gain per day. Feed conversion ratio (FCR) was calculated as the ratio of FI and body weight gain. At 58 D of age, broilers were harvested in accordance with humane and commercial slaughter procedures. End live weight was taken immediately before slaughter. After exsanguination, plucking, and

evisceration, empty hot carcass weights were taken. Carcasses were then chilled for 1 h in an ice water bath. After the carcasses were chilled in a walk-in cold room at 5°C overnight, chilled carcass weights were recorded, and the left P. major muscle was weighed. P. major weight was calculated as twice the weight of the left P. major muscle. Right P. major muscles were frozen in a –30°C freezer for further analysis.

Meat Color and Ultimate pH Analysis

Meat color and ultimate pH were evaluated on the left P. major muscle. Meat color was measured using a CR-410 Chroma Meter (Konica Minolta Sensing, Singapore) according to the procedure by Fletcher (1999). The color of each breast fillet was reported by values of L* (lightness), a* (redness), and b* (yellowness). Ultimate pH was evaluated as previously described by Lyon et al. (1985) with a portable pH meter (Model HI98249, Hanna Instruments, Woonsocket, RI). Each sample was measured in triplicate, and the average pH value was calculated for each sample. After pH was measured, the P. major muscles were frozen in a –30°C freezer.

Thaw Loss, Cooking Loss, and Tenderness Analysis

Frozen breast muscles were thawed at 4°C for 24 h. Thawed breast muscles were weighed, and thaw loss

Table 2. Feed ingredients and calculated nutritional composition of grower diets.¹

Item	Control	Grower VE	Grower n-3	Grower VE and n-3
Ingredients, % as-fed				
Corn	56.28	50.33	50.35	50.33
Soybean meal	28.10	33.71	33.71	33.71
Poultry byproduct meal	7.50	7.50	7.50	7.50
Sodium chloride	0.23	0.22	0.22	0.22
Limestone	1.02	1.10	1.10	1.10
Dicalcium phosphate	0.29	0.47	0.47	0.47
Premix ² (Akey #7)	0.35	0.35	0.35	0.35
L-Lys HCl	0.15	0.15	0.15	0.15
DL-Met	0.30	0.34	0.34	0.34
L-Thr	0.09	0.11	0.11	0.11
NaHCO ₃	0.10	0.10	0.10	0.10
Selenium	0.10	0.10	0.10	0.10
Amprolium	1.00	1.00	1.00	1.00
DL- α -tocopherol acetate	0.001	0.020	0.001	0.021
Soy oil	0.17	0.17	1.87	1.87
Corn oil	3.81	3.81	0.11	0.11
Fish oil	-	-	2.53	2.53
Hydrogenated coconut oil	0.52	0.52	-	-
Calculated nutrients and energy				
AME, kcal/kg	3,107	3,106	3,110	3,109
Protein, %	21.56	21.56	21.56	21.56
Calcium, %	0.87	0.87	0.87	0.87
Available phosphorus, %	0.43	0.43	0.43	0.43
Digestible Lys, %	1.15	1.15	1.15	1.15
Digestible Met + Cys, %	0.87	0.87	0.87	0.87

¹Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of VE, n-3 fatty acids, or combination of both was performed during the grower phase (11–24 D). Dietary treatments during the starter phase (0–10 D) received the same diets as the control group during the grower phase.

²The premix provides the following per kg of diet: vitamin A, 1,715,000 IU; vitamin D3, 6,000 IU; vitamin E, 12,600 IU; vitamin B12, 11 μ g; folic acid, 1.5 mg; biotin, 150 μ g; calcium carbonate, 25 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; and ethoxyquin, 150 mg (Provimi North America, Brookville, OH).

was determined by calculating the weight before freezing and after thawing. Thermometers were placed at the center of the breast muscles to monitor their

temperature. Muscles were placed in a 150°C preheated oven and cooked until internal temperature reached 77°C. Muscles were cooled to room temperature and

Table 3. Feed ingredients and calculated nutritional composition of finisher diets.¹

Item	Finisher 1 diets (25–38 D)	Finisher 2 diets (39–51 D)	Finisher 3 diets (52–58 D)
Ingredients, % as-fed			
Corn	61.23	64.23	66.68
Soybean meal	22.98	20.35	18.35
Poultry byproduct meal	7.50	7.50	7.50
Sodium chloride	0.23	0.23	0.23
Limestone	0.94	0.91	0.88
Dicalcium phosphate	0.10	0.02	-
Premix ² (Akey #7)	0.35	0.35	0.35
L-Lys HCl	0.13	0.11	0.12
DL-Met	0.27	0.23	0.22
L-Thr	0.07	0.06	0.05
NaHCO ₃	0.10	0.10	0.10
Selenium	0.10	0.10	0.10
Amprolium	1.00	1.00	1.00
Corn oil	3.00	2.71	2.40
Blended fat	2.00	2.10	2.02
Calculated nutrients and energy			
AME, kcal/kg	3,203	3,225	3,226
Protein, %	19.58	18.60	17.87
Calcium, %	0.78	0.74	0.72
Available phosphorus, %	0.39	0.37	0.36
Digestible Lys, %	1.02	0.95	0.91
Digestible Met + Cys, %	0.80	0.74	0.71

¹Broilers in all experimental groups were fed with same finisher diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids.

²The premix provides the following per kg of diet: vitamin A, 1,715,000 IU; vitamin D3, 6,000 IU; vitamin E, 12,600 IU; vitamin B12, 11 μ g; folic acid, 1.5 mg; biotin, 150 μ g; calcium carbonate, 25 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; and ethoxyquin, 150 mg (Provimi North America, Brookville, OH).

Table 4. Analyzed fatty acid composition of starter and grower diets.¹

Item, %	Starter diets				Grower diets			
	Control	VE	n-3	VE and n-3	Control	VE	n-3	VE and n-3
C _{8:0}	0.18	0.00	0.00	0.00	0.00	0.28	0.00	0.00
C _{10:0}	0.28	0.20	0.00	0.00	0.20	0.24	0.00	0.00
C _{12:0}	2.14	1.56	0.00	0.00	1.59	1.84	0.00	0.00
C _{14:0}	0.99	0.74	1.57	1.83	0.62	0.82	1.74	1.80
C _{15:0}	0.00	0.00	0.13	0.25	0.00	0.00	0.15	0.21
C _{16:0}	14.18	11.48	13.26	14.74	11.34	11.50	14.76	14.44
C _{16:1}	0.45	0.39	2.58	2.84	0.31	0.37	0.15	2.72
C _{17:0}	0.00	0.00	0.20	0.36	0.00	0.05	0.32	0.29
C _{17:1}	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00
C _{18:0}	2.49	2.59	3.95	3.16	2.89	2.91	3.60	3.86
C _{18:1}	29.44	26.14	20.20	19.78	26.16	25.08	21.13	20.75
C _{18:2} (n-6)	46.18	53.79	41.37	41.53	54.02	54.09	43.91	40.96
C _{18:3} (n-6)	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
C _{18:3} (n-3)	2.26	1.82	3.67	3.64	1.79	1.77	3.49	3.40
C _{20:0}	0.45	0.40	0.40	0.34	0.37	0.37	0.32	0.36
C _{20:1}	0.29	0.27	0.47	0.39	0.24	0.23	0.40	0.40
C _{20:2}	0.00	0.00	0.08	0.16	0.00	0.04	0.15	0.13
C _{20:4} (n-6)	0.17	0.17	0.48	0.43	0.13	0.06	0.40	0.41
C _{20:3} (n-3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C _{20:5} (n-3)	0.00	0.00	4.37	4.05	0.00	0.00	3.81	4.00
C _{22:0}	0.26	0.20	0.38	0.31	0.17	0.14	0.22	0.34
C _{22:5} (n-3)	0.00	0.00	0.90	0.78	0.00	0.00	0.66	0.74
C _{22:6} (n-3)	0.00	0.00	5.63	4.93	0.00	0.00	4.49	4.91
C _{24:0}	0.24	0.25	0.37	0.29	0.17	0.22	0.25	0.29
SFA ²	21.20	17.42	20.26	21.29	17.34	18.36	21.36	21.58
MUFA ³	29.73	26.41	20.67	20.37	26.41	25.31	21.53	21.15
PUFA ⁴	48.61	55.78	56.50	55.51	55.93	55.96	56.96	54.55
n-3	2.26	1.82	14.57	13.39	1.79	1.77	12.47	13.06
n-6	46.35	53.96	41.85	41.96	54.15	54.15	44.34	41.37
n-6:n-3	20.5	29.6	2.9	3.1	30.3	30.6	3.6	3.2

¹Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D) or grower phase (11–24 D).

²SFA = Saturated fatty acids.

³MUFA = Monounsaturated fatty acids.

⁴PUFA = Polyunsaturated fatty acids.

weighed as a final cooling weight, which was used to calculate cooking loss. Cooking loss was based on the difference between weight after thawing and weight after cooking. The breast muscles were then used for tenderness analysis (Blunt-Meullenet-Owens Razor Shear force) according to the procedure of Lee et al. (2008). Each breast muscle was sheered in 5 locations by the blade penetrating the breast perpendicular to the direction of the breast muscle fibers with a TA-XT Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Shear force and shear energy were recorded to evaluate meat tenderness.

Moisture and Fat Analysis

The right P. major muscle was used to determine percentage of moisture and intramuscular fat content. A total of 210 samples with 10 replicates with 3 samples per replicate were used. The muscle samples were thawed and ground in a food processor until samples were completely homogenous. Each sample was placed in the aluminum pan and weighed. After drying in a 110°C oven for 48 h, the samples were cooled in a desiccator to room temperature and weighed. Moisture content was calculated based on weight before and after

drying. Ether extraction was conducted in AnkomXT 15 extractor (Ankom Technology, Macedon, NY) with filter paper bag holding the dried samples following the instructions of the manufacturer (Ankom Technology). The filter paper bag was placed in an oven at 110°C for 24 h after extraction and was weighed. Fat content was calculated based on weight before and after the ether extraction.

Wooden Breast and White Striping Scores

A total of 210 samples with 10 replicates with 3 samples per replicate were used to evaluate WB and WS scores. Wooden breast and WS were scored by observation and palpation of the left P. major muscle after the carcass was sufficiently chilled. Wooden breast scores were based on palpable firmness using a 0 to 3 scale as described by Tijare et al. (2016) with a score of 0 meaning normal firmness and a score of 3, indicating the most firm. Scores of 1 and 2 were intermediate. White striping scores were based on white striations as described by Kuttappan et al. (2012b). A score of 0 indicated no WS with no white striations and a score of 3 severe WS with a high level of white striations. Scores of 1 and 2 were intermediate.

Table 5. Analyzed fatty acid composition of finisher diets.¹

Item, %	Finisher 1 diets (25–38 D)	Finisher 2 diets (39–51 D)	Finisher 3 diets (52–58 D)
C _{8:0}	0.00	0.00	0.00
C _{10:0}	0.95	0.00	0.00
C _{12:0}	0.00	0.00	0.00
C _{14:0}	0.17	0.13	0.14
C _{15:0}	0.00	0.01	0.00
C _{16:0}	12.96	13.81	11.19
C _{16:1}	0.68	0.38	0.58
C _{17:0}	0.00	0.00	0.00
C _{17:1}	0.00	0.00	0.00
C _{18:0}	3.24	2.03	1.75
C _{18:1}	28.37	18.09	25.20
C _{18:2} (n-6)	50.28	62.13	47.56
C _{18:3} (n-6)	0.00	0.00	10.54
C _{18:3} (n-3)	1.96	2.15	1.87
C _{20:0}	0.36	0.36	0.31
C _{20:1}	0.28	0.19	0.25
C _{20:2}	0.13	0.11	0.00
C _{20:4} (n-6)	0.24	0.23	0.22
C _{20:3} (n-3)	0.00	0.00	0.00
C _{20:5} (n-3)	0.00	0.00	0.00
C _{22:0}	0.18	0.18	0.18
C _{22:5} (n-3)	0.00	0.00	0.00
C _{22:6} (n-3)	0.00	0.00	0.00
C _{24:0}	0.19	0.20	0.20
SFA ²	18.07	16.72	13.77
MUFA ³	28.66	18.28	25.46
PUFA ⁴	52.60	64.62	60.19
n-3	1.96	2.15	1.87
n-6	50.51	62.36	58.32
n-6:n-3	25.8	29.0	31.2

¹Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D) or grower phase (11–24 D).

²SFA = Saturated fatty acids.

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⁴PUFA = Polyunsaturated fatty acids.

Statistical Analysis

Data were analyzed as a completely randomized design using PROC MIXED procedure of SAS, version 9.4 software. Individual pen was identified as the experimental unit. Dietary treatments were used as a fixed effect. Least square means were estimated with the LSMEANS procedure and separated with the PDIF option. Data from VE supplementation treatments both in starter and grower phases was combined to determine VE effect by setting up orthogonal contrasts. Effect of n-3 fatty acids was determined by setting up orthogonal contrasts using data from n-3 fatty acids supplementation treatments both in starter and grower phases. Data from VE and n-3 fatty acids supplementation treatments both in starter and grower phases were combined to determine combination effect by setting up orthogonal contrasts. Significance was accepted at $P \leq 0.05$.

RESULTS

Growth Performance

Growth performance of the broilers in this study is shown in Table 6. During the starter phase (0–10 D), broilers fed with different dietary treatments did not have a significant difference in FI ($P = 0.79$) or FCR

($P = 0.40$). However, broilers fed with increased dietary n-3 fatty acids (n-6/n-3 ratio of 3:1) in the starter diets had lower ADG compared with the control group ($P = 0.049$). Broilers supplemented with increased levels of n-3 fatty acids (n-6/n-3 ratio of 3:1) in starter diets had lower ADG than supplemented in grower diets during starter phase ($P = 0.016$). Additionally, supplemental VE (200 IU/kg) in the grower diets had a decreased FCR ($P = 0.035$). Neither FI ($P = 0.59$) nor FCR ($P = 0.42$) was different among the dietary groups during the finisher phase (25–58 D). Nevertheless, broilers in the starter n-3 group had decreased ADG than the control group ($P = 0.040$) and the grower n-3 group ($P = 0.011$) during the finisher phase. No significant effect of VE, n-3 fatty acids, or combination of both was shown on broiler growth performance during the entire experiment ($P > 0.05$). However, there was a trend that increased VE supplementation decreased FCR in grower phase ($P = 0.06$) while n-3 fatty acids increased FCR in finisher phase ($P = 0.09$).

Meat Yield

No significant effect of VE, n-3 fatty acids, or the combination of both was shown on broiler meat yield ($P > 0.05$; Table 7). However, n-3 fatty acids supplementation (n-6/n-3 ratio of 3:1) in the starter diets significantly decreased final body weight ($P = 0.024$),

Table 6. Effect of vitamin E and omega-3 fatty acids on growth performance of broilers.¹

Item	Treatments							P-value			
	Control	Starter VE	Starter n-3	Starter VE and n-3	Grower VE	Grower n-3	Grower VE and n-3	SEM ²	VE effect	n-3 effect	VE and n-3 effect
Starter phase (0–10 D)											
ADG ³	15.71 ^{a,b,c}	16.42 ^a	14.03 ^d	14.61 ^{a,b,c,d}	14.72 ^{a,b,c,d}	16.41 ^{a,b}	14.64 ^{a,b,c,d}	0.20	0.84	0.49	0.14
FI ⁴	276.87	268.39	274.79	277.79	271.96	275.85	242.18	3.85	0.69	0.93	0.33
FCR ⁵	0.99	0.92	1.14	1.09	1.06	0.97	0.96	0.02	0.99	0.45	0.73
Grower phase (10–24 D)											
ADG	38.65	39.38	36.68	39.78	39.29	38.37	36.61	0.50	0.65	0.46	0.77
FI	1019.96 ^{a,b}	1019.57 ^{a,b}	934.24 ^{a,b}	1064.80 ^b	899.84 ^b	1009.47 ^{a,b}	968.65 ^{a,b}	10.74	0.25	0.36	0.95
FCR	1.82 ^{a,b}	1.70 ^{a,b,c}	1.73 ^{a,b,c}	1.86 ^a	1.58 ^c	1.77 ^{a,b,c}	1.81 ^{a,b,c}	0.03	0.06	0.48	0.88
Finisher phase (25–58 D)											
ADG	80.73 ^{b,c}	76.59 ^a	75.34 ^d	82.69 ^{b,c,d}	78.29 ^{b,c,d}	83.12 ^b	80.26 ^{b,c,d}	0.77	0.13	0.49	0.74
FI	4199.81	4081.89	4221.88	4541.95	4261.92	4489.25	4263.25	38.22	0.87	0.39	0.29
FCR	1.52	1.52	1.62	1.60	1.59	1.58	1.53	0.02	0.42	0.09	0.33

^{a-d}Values within a row without a common letter are significantly different ($P \leq 0.05$).

¹Values are means of 10 pens per treatment, each pen included 3 birds. Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of extra VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D) or grower phase (11–24 D).

²SEM = Standard error of the mean.

³ADG = Average daily gain (g).

⁴FI = Feed intake (g).

⁵FCR = Feed conversion ratio.

hot carcass weight ($P = 0.049$), and chilled carcass weight ($P = 0.024$) of broilers compared with the control group. Broilers supplemented with increased levels of both VE (200 IU/kg) and n-3 fatty acid (n-6/n-3 ratio of 3:1) in starter diets did not show a difference in meat yield compared with control group ($P > 0.05$). There was no significant difference in P. major muscle weight among each group ($P = 0.90$).

Meat Color and Ultimate pH

The meat quality data are presented in Table 8. Broilers fed a starter diet with both VE (200 IU/kg) and n-3 fatty acids (n-6/n-3 ratio of 3:1) had decreased L* (lightness) values compared with the control group ($P = 0.027$), starter VE ($P = 0.050$), and starter n-3 group ($P = 0.045$). Supplemental VE (200 IU/kg) and n-3 fatty acids (n-6/n-3 ratio of 3:1) in the grower diets increased b* (yellowness) in the breast muscle compared with supplementing with n-3 fatty acids in the grower diets ($P = 0.039$). There was no significant difference in pH value among the different dietary treatments ($P = 0.60$).

and no significant effect of VE, n-3 fatty acids, or combination of both on meat quality ($P > 0.05$).

Thaw Loss, Cooking Loss, and Tenderness

The combination of both VE (200 IU/kg) and n-3 fatty acids (n-6/n-3 ratio of 3:1) in the starter diets significantly increased shear force compared with the control group ($P = 0.046$). Breast muscle from the starter VE group had a lower shear force than the grower VE group ($P = 0.049$). No significant difference was shown on thaw loss, cook loss, or shear energy in any group compared with the control group ($P > 0.05$).

Moisture and Fat Content

Results of the proximate analysis are shown in Table 9. Breast muscle from the starter VE and n-3 group contained lower moisture content compared with the other groups ($P \leq 0.05$). The breast muscle of broilers supplemented with an increased concentration of VE (200 IU/kg) in the starter diets or grower diets had lower fat content than the breast muscle from the

Table 7. Effect of vitamin E and omega-3 fatty acids on meat yield of broilers.¹

Item	Treatments							P-value			
	Control	Starter VE	Starter n-3	Starter VE and n-3	Grower VE	Grower n-3	Grower VE and n-3	SEM ²	VE effect	n-3 effect	VE and n-3 effect
Final body weight	3593.56 ^a	3466.45 ^{a,b}	3344.91 ^b	3660.58 ^a	3484.78 ^{a,b}	3571.41 ^{a,b}	3521.81 ^{a,b}	33.00	0.20	0.14	0.98
Hot carcass weight	2707.99 ^a	2602.52 ^{a,b}	2537.79 ^b	2800.40 ^a	2650.02 ^{a,b}	2694.61 ^{a,b}	2663.03 ^{a,b}	26.42	0.26	0.21	0.75
Chilled carcass weight	2792.27 ^a	2691.53 ^{a,b}	2585.96 ^b	2888.32 ^a	2727.37 ^{a,b}	2799.75 ^a	2739.22 ^{a,b}	27.60	0.28	0.20	0.78
P. major weight ³	662.46	641.77	649.23	689.32	665.32	672.58	659.53	8.76	0.72	0.95	0.64

^{a, b}Values within a row without a common letter are significantly different ($P \leq 0.05$).

¹Values are means of 10 pens per treatment, each pen included 3 birds. Meat yield was measured at the end of the study. Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of extra VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D) or grower phase (11–24 D).

²SEM = Standard error of the mean.

³Pectoralis major (P. major) muscle weight was calculated as the 2 times the weight of the left P. major muscle.

Table 8. Effect of vitamin E and omega-3 fatty acids on meat quality of broilers.¹

Item	Treatments							SEM ²	P-value		
	Control	Starter VE	Starter n-3	Starter VE and n-3	Grower VE	Grower n-3	Grower VE and n-3		VE effect	n-3 effect	VE and n-3 effect
l ³	64.11 ^a	64.07 ^a	64.10 ^a	62.61 ^b	63.75 ^{a,b}	63.89 ^{a,b}	63.94 ^{a,b}	0.18	0.70	0.83	0.11
a ³	12.93	12.53	12.96	13.22	12.88	12.85	12.55	0.10	0.44	0.92	0.87
b ³	12.89 ^{a,b}	12.38 ^b	13.35 ^{a,b}	12.81 ^{a,b}	12.91 ^{a,b}	12.49 ^b	13.90 ^a	0.16	0.62	0.95	0.35
pH	5.79	5.79	5.82	5.79	5.79	5.80	5.82	0.01	0.99	0.22	0.52
Thaw loss (%)	1.78	1.61	1.45	1.47	1.61	1.54	1.68	0.08	0.44	0.20	0.38
Cook loss (%)	19.59	19.90	18.76	19.21	20.41	19.08	19.95	0.36	0.57	0.51	0.99
Shear force (N)	14.74 ^b	14.70 ^b	14.86 ^{a,b}	15.95 ^a	15.78 ^a	14.16 ^b	14.88 ^{a,b}	0.17	0.29	0.64	0.17
Shear energy (N/mm)	1.87	1.92	1.86	1.97	1.96	1.83	1.85	2.05	0.17	0.74	0.42

^{a-c}Values within a row without a common letter are significantly different ($P \leq 0.05$).

¹Values are means of 10 pens per treatment, each pen included 3 birds. Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of extra VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D) or grower phase (11–24 D).

²SEM = Standard error of the mean.

³L* means lightness, a* means redness, and b* means yellowness.

starter n-3 group ($P = 0.017$). Other than these significant instances, there were no other significant effects of VE, n-3 fatty acids, or combination of both on moisture and fat content ($P > 0.05$).

Wooden Breast and White Striping Scores

Distribution analysis of the WB and WS scores from the different dietary groups is shown in Figure 1. In the control group, the distribution of WB scoring was with no WB 18.37%, with a mild score of one 48.98%, with a moderate score of two 24.49%, and with a severe score of three 8.16%. Supplemental VE in the starter diets significantly increased the percentage of P. major muscles with no WB, score of 0 to 48.00%. Thus, supplemental VE in the started diet decreased the severity of WB (Figure 1A). The higher concentration of VE (200 IU/kg) in the starter diets had a greater effect on reducing WB score than in the grower diets. In contrast, n-3 fatty acid (n-6/n-3 ratio of 3:1) in the starter or grower diets increased the severity of WB with moderate and severe scores of 2 and 3, respectively. However, combination of VE and n-3 fatty acids in either the starter or grower diets decreased the severity of WB compared with supplementation of n-3 fatty acids alone in the diets.

The percentage of WS was also modified by the diets. There was a higher percentage of no WS (score of 0) and mild WS (score of 1) and a reduction in the percentage of moderate (score of 2) and severe (score of 3) WS (Figure 1B), when broilers were fed with the higher concentration of VE (200 IU/kg) in the starter or grower diets. The starter VE group showed more of an effect on reducing severity of WS compared with the grower VE group. In contrast, n-3 fatty acids increased the severity of WS in both the starter and grower diets.

DISCUSSION

The present study compared the effects of VE, n-3 fatty acid, and combination of both during the starter phase (0–10 D) or grower phase (11–24 D) on growth performance, meat yield, meat quality, and severity of WB

and WS. Oxidative stress (Mutryn et al., 2015; Abasht et al., 2016) and inflammation (Mudalal et al., 2015; Sihvo et al., 2017) have been found in WB affected breast muscle by histological changes and genomic analysis. Vitamin E and n-3 fatty acid dietary supplementation was selected because of their antioxidant and anti-inflammatory properties, respectively.

In general, VE supplementation did not impact growth performance and meat yield. These findings were in agreement with previous studies in which neither body weight nor meat yield were affected by dietary VE (Bartov and Frigg, 1992; Sakamoto et al., 2006; Kuttappan et al., 2012b; Cheng et al., 2018). This could be because of the fact that the contents of VE in corn–soybean meal basal diets were sufficient to meet the requirement for growth performance and meat production of broilers and would not be changed by increased concentrations of VE supplementation. Omega-3 fatty acids, on the other hand, had a negative influence on growth performance and meat yield, especially when the concentration was increased in starter diets, which was consistent with earlier studies (Ayerza et al., 2002; Azcona et al., 2008; Navidshad, 2009). Similar results were reported by Hulan et al. (1988) that fish oil–enriched diets resulted in lower body weight and higher FCR. They suggested that the poorer growth performance was because of lower palatability and higher calcium levels. It was also reported that PUFA reduced *de novo* synthesis resulting in decreased fat deposition (Smink et al., 2010). This finding may explain the reduction in meat yield when n-3 fatty acids were increased in the diet because meat yield is dependent on muscle amount in relation to fat deposit. Several studies using fish oil in the diets, however, did not find adverse effects on body weight or FCR (Lopez-Ferrer et al., 2001; Farhoomand and Checaniazzer, 2009). The contrasting results could be related to the concentration of PUFA. Higher PUFA level is more likely to have an adverse effect on growth performance and meat yield. When increased concentrations of VE were combined with n-3 fatty acids, both growth performance and meat yield had no significant change

Table 9. Effect of vitamin E and omega-3 fatty acids on moisture and fat contents of breast muscle.¹

Item (%)	Treatments							SEM ²	P-value		
	Control	Starter VE	Starter n-3	Starter VE and n-3	Grower VE	Grower n-3	Grower VE and n-3		VE effect	n-3 effect	VE and n-3 effect
Moisture	75.75 ^a	75.61 ^a	75.69 ^a	75.10 ^b	75.69 ^a	75.63 ^a	75.93 ^a	0.06	0.56	0.61	0.18
Fat	1.39 ^{a,b}	1.30 ^b	1.50 ^a	1.47 ^{a,b}	1.34 ^b	1.43 ^{a,b}	1.43 ^{a,b}	0.02	0.25	0.23	0.40

^{a, b}Values within a row without a common letter are significantly different ($P \leq 0.05$).

¹Values are means of 10 pens per treatment, each pen included 3 birds. Broilers in control group were fed with diets with standard levels of vitamin E (VE) and omega-3 (n-3) fatty acids during the entire study (0–58 D). Supplementation of extra VE, n-3 fatty acids, or combination of both was performed during the starter phase (0–10 D) or grower phase (11–24 D).

²SEM = Standard error of the mean.

compared with the control group. Voljč et al. (2011) found that VE reduced lipid peroxidation in meat and therefore reduced the negative effect on meat yield.

Meat quality is closely related with muscle growth and development. Postnatal muscle growth is dependent on satellite cells, which fuse with multinucleated myofibers, donate their nuclei, and increase protein synthesis capabilities (Moss and Leblond, 1971). This results in muscle growth through hypertrophy or the enlargement of existing muscle fibers. Satellite cells are most active the first week posthatch (Halevy et al., 2000; Mozdziaik et al., 2002). They are sensitive to nutritional and environmental changes and will have long-term effects on muscle growth and meat quality (Dangott et al., 2000; Halevy et al., 2001; Velleman et al., 2014). Sufficient nutrients during the first week posthatch are critical for maximal muscle growth (Halevy et al., 2001; Powell et al., 2014), whereas nutrient restriction during the first week posthatch influences myogenic genes expression and fat deposition in broilers (Velleman et al., 2010, 2014; Powell et al., 2014). Therefore, early posthatch nutritional strategies can be used to modify satellite cells and influence muscle structure and meat quality.

Meat color is an important indicator of meat quality. There were no significant differences in a* (redness) in the current study. Lu et al. (2014) reported similar results in their VE treatment in broiler chickens. L* (lightness) decreased when increased VE and n-3 fatty acids were added in starter diets, which is similar to the findings of Cheng et al. (2016). Their study attributed the discoloration of meat to lipid peroxidation. Meat tenderness is another important indicator of meat quality, which depends on a number of factors such as collagen, water, and lipid content. Shear force, commonly used to show meat tenderness, was lower when VE was supplemented in starter diets than in grower diets in the current study. Meanwhile, fat content in broilers supplemented with VE in starter group was lower than starter n-3 supplementation group. Papah et al. (2017) showed fat content increased when degenerated muscle fibers were replaced by adipose tissue. Broilers supplemented with VE in the starter diets had lower fat content in the current study, suggesting dietary VE supplementation in during the starter phase may have the potential to reduce muscle fiber degeneration.

Wooden breast and WS were reduced when VE was increased in starter or grower diets of the broilers. The

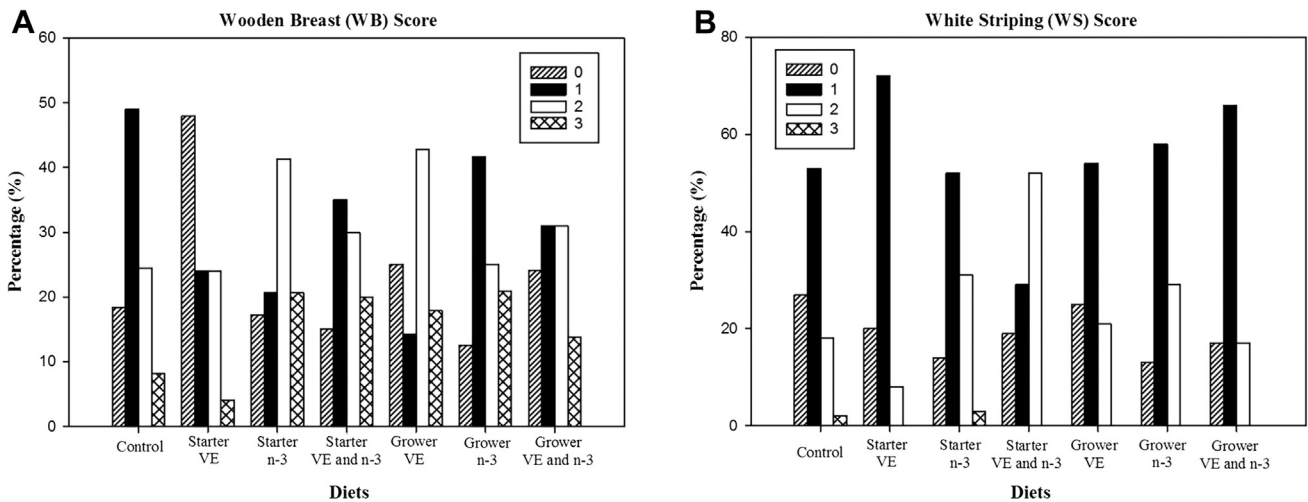


Figure 1. Effect of vitamin E (VE) and omega-3 (n-3) fatty acids on wooden breast (WB) score (A) and white striping (WS) score (B) of the breast muscle. A total of 210 breast muscles with 10 replicates of 3 muscles per replicate were evaluated. Broilers in the control group were fed with corn-soybean meal basal diet with VE (10 IU/kg) and n-3 fatty acids (n-6/n-3 ratio of 30:1) at a standard level during the entire study (0–58 D). Starter VE, starter n-3, starter VE and n-3 were supplemented with VE (200 IU/kg), n-3 fatty acids (n-6/n-3 ratio of 3:1), or a combination of both during the starter phase (0–10 D). Grower VE, grower n-3, grower VE and n-3 were supplemented with VE (200 IU/kg), n-3 fatty acids (n-6/n-3 ratio of 3:1), or a combination of both during the grower phase (11–24 D). Scores of WB and WS were based on palpation and visual observation. Score 0 = none, 1 = mild, 2 = moderate, 3 = severe.

effect of VE on reducing the myopathies could be because of its antioxidant potential. Several studies have indicated that WB affected broilers are under severe oxidative stress (Mutryn et al., 2015; Abasht et al., 2016) and inflammation (Sihvo et al., 2014; Mudalal et al., 2015). Oxidative stress is considered an imbalance between oxidants and antioxidants (Voljč et al., 2011). Oxygen can be reduced to water during respiration. If oxygen cannot be reduced completely, reactive oxygen species with high oxidizing power will be produced. Oxidative stress occurs when the antioxidant system of an individual cannot remove reactive oxygen species properly (Panda and Cherian, 2014). The presence of oxidative stress in WB condition could be because of insufficient vascularization, as larger diameter myofibers found frequently in heavy weight fast growing broilers restrict the space for capillaries (Velleman and Nestor, 2003).

Oxidative stress could be reduced by antioxidant scavenging free radicals (Miller et al., 1993). Vitamin E is a very powerful antioxidant known to prevent cells and tissues from oxidative damage (Voljč et al., 2011). Among the 8 forms of VE, DL- α -tocopherol acetate is the form commonly used in poultry industry and has a high biological efficiency (Panda and Cherian, 2014). The high efficiency comes from incorporation of α -tocopherol directly into cell membranes where oxidation is initiated (Bou et al., 2009). Therefore, myopathies can be reduced by VE through their highly efficient antioxidant effect. Meanwhile, satellite cells have the highest mitotic activity the first week posthatch (Halevy et al., 2000; Mozdziaik et al., 2002), which may explain why extra VE supplementation in starter diets had a better effect on improving tenderness while reducing the severity of WB and WS compared with the grower diets. Additionally, there was no significant effect of VE, n-3 fatty acids, or combination of both on growth, meat yield, and meat quality. But significant differences were found within each dietary treatment in many parameters, indicating the importance of supplementation time.

In contrast, n-3 fatty acids did not show a beneficial effect on reducing the myopathies. Omega-3 fatty acids, including α -linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3), are essential fatty acids. They have been shown to exert anti-inflammatory effects through altering proinflammatory cytokines and adhesion molecules (Simopoulos, 2002; Calder, 2003; Rahimi et al., 2011; Yu et al., 2018). However, there is no reported beneficial effect on improving meat quality and reducing severity of WB (Lopez-Ferrer et al., 2001). The combination of VE and n-3 fatty acids in the starter diet did not have a greater effect on reducing WB compared with vitamin E supplementation alone or the control group. Nevertheless, the combination of VE and n-3 fatty acids did decrease the severity of WB compared with n-3 fatty acids supplementation alone.

In conclusion, VE supplementation reduced severity of the WB myopathy without sacrificing growth

performance and meat yield. Supplemental VE during the starter phase had a better effect on improving meat quality and reducing WB than supplementation during the grower phase. Omega-3 fatty acids supplementation in starter diets, however, reduced growth performance and meat yield without showing beneficial effect on reducing WB. Combination of VE and n-3 fatty acids decreased the negative impact of n-3 fatty acids on growth performance and meat yield. Future research needs to be focused on determining the effect of VE on muscle structure and gene expression involved in muscle growth, oxidative stress, and inflammation.

ACKNOWLEDGMENTS

This study was supported by US Poultry and Egg grant (project #710) to SGV and SKJ and the China Scholarship Council (No. 201706350026) to JW. The authors would like to thank Janet McCormick for technical assistance.

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