Research Note: Dietary phytase reduces broiler woody breast severity via potential modulation of breast muscle fatty acid profiles

Reagan N. Cauble,[*,2] Elizabeth S. Greene,[†] Sara Orlowski,[†] Carrie Walk,[‡,1] Mike Bedford,[‡] Jason Apple,[*] Michael T. Kidd,[†] and Sami Dridi[†,2]

[*]*Department of Animal Science, University of Arkansas, Fayetteville 72701;* [†]*Center of Excellence for Poultry Science, Division of Agriculture, University of Arkansas, Fayetteville 72701; and* [‡]*AB Vista, Woodstock Ct, Marlborough, Wiltshire SN8 4AN, UK*

ABSTRACT Woody breast (**WB**) myopathy is a major concern and economic burden to the poultry industry, and for which, there is no effective solution because of its unknown etiology. In a previous study, we have shown that phytase (Quantum Blue, **QB**) reduces the WB severity by 5% via modulation of oxygen homeostasis-related pathways. As WB has been suggested to be associated with lipid dysmetabolism, we aimed to determine the effect of QB on WB and breast muscle fatty acid profile. Male broilers were subjected to 6 treatments (96 birds/treatment): a nutrient adequate control group (PC), the PC supplemented with 0.3% myo-inositol (PC + MI), a negative control (**NC**) deficient in available P and Ca by 0.15 and 0.16%, respectively, the NC fed with QB at 500 (NC+500 FTU), and 1,000 (NC+ 1,000 FTU) or 2,000 FTU/kg of feed (NC+2,000 FTU). Woody breast and white striping scores were recorded, and fatty acid profiles were determined using gas liquid chromatography. Woody breast-affected muscles exhibited a significant higher incidence of white striping as liquid chromatography analysis reveals an imbalance of fatty acid profile in the breast of WB-affected birds with a significant higher percent of saturated fatty acids (SFA, myristic [14:0], pentadecanoic [15:0], and margaric [17:0]) and monounsaturated fatty acids (myristoleic [14:1], palmitoleic [16:1*c*], 10-trans-heptadecenoic [17:1*t*], oleic [18:1*c*9], and vaccenic [18:1*c*11]), and lower content of polyunsaturated fatty acids (PUFA) and omega-3 ($P < 0.05$). Quantum Blue at high doses (1,000 and 2,000 FTU) significantly reduces the percent of SFA and increases that of PUFA compared with the control group. In conclusion, WB myopathy seemed to be associated with an imbalance of fatty acid profile, and QB ameliorates the severity of WB potentially via modulation of SFA and PUFA contents.

Key words: fatty acid profile, phytase, woody breast, white striping, chickens

2020 Poultry Science 99:4009–4015
https://doi.org/10.1016/j.psj.2020.05.005

INTRODUCTION

Woody breast (**WB**) is one of the most important myopathies currently challenging the poultry industry (Bowker, 2016; Picchi, 2016). It was first described in 2014 and was characterized by visually hard, outbulging, and pale areas on the ventral surface of *pectoralis major* muscle (Sihvo et al., 2014). The phenotypic stiffness of WB occurs in varying degrees and significantly affects quality traits and functional properties of broiler breast muscle (Mudalal et al., 2015; Soglia et al., 2016). Woody breast has emerged on a global scale with documented occurrences in the United States (Kuttappan et al., 2016), Finland (Sihvo et al., 2014), Italy (Mudalal et al., 2015), the United Kingdom (de Brot et al., 2016), and in many other countries, with incidences reportedly affecting broilers from 30 to 50% that are grown for 8 wk to a live body weight greater than 4.2 kg. The myopathy constitutes a major animal health, welfare, and economic concern and is estimated to cost the US poultry industry more than 200 million dollars a year because of on-farm culling and mortality, downgrading, and condemnation at processing, as well as rejection from human consumption (Kuttappan et al., 2016).

Received October 17, 2019.
Accepted May 17, 2020.

[1]Present address: DSM Nutritional Products, Heanor DE75 7SY, UK.
[2]Corresponding authors: rncauble@uark.edu (RNC); dridi@uark.edu (SD)

Although the etiology of the myopathy is still unknown, histological evidence of the hemorrhagic lesions indicated multifocal degeneration and necrosis of muscle tissue with infiltration of inflammatory cells, connective tissues, and fat (Sihvo et al., 2014).

Recently, we provided mechanistic evidence that WB myopathy is associated with systemic and local breast muscle hypoxia, and we found that dietary phytase (Quantum Blue, **QB**) supplementation reduce the severity of WB by 5% (Greene et al., 2019). Although dietary phytase enzyme is accepted in practice for the consistent intestinal degradation of plant phytate and improvement of phosphorus digestibility and feed efficiency (Singh, 2008), QB has been recently shown to reduce WB severity via improvement of oxygen homeostasis (Greene et al., 2019). As hypoxic conditions have been reported to limit the regenerative capacity of muscle fibers by favoring the replacement of degenerated muscle fibers with lipid and fibrotic tissues (Hoppeler and Vogt, 2001) and as QB ameliorates hypoxia, we hypothesized that 1) breast muscle fatty acid profile could be different between WB-affected and unaffected broilers and 2) QB may modulate fatty acid profile. The present study was, therefore, undertaken to answer these questions.

MATERIALS AND METHODS

Care and Use of Animals

The present study was conducted in accordance with the National Institutes of Health recommendations guide for laboratory animal use and care. All the procedures in this study were approved by the University of Arkansas Animal Care and Use Committee under protocol #16084.

Animals, Diet, and Experimental Design

The experimental design has been previously described (Greene et al., 2019). Briefly, day-old male broiler chicks (Cobb 500, n = 576) were weighed and assigned randomly to 48 floor pens (12 birds/pen). Pens were covered with clean pine wood shavings and equipped with separate feeders and water lines in a controlled environment. Ambient temperature was gradually reduced from 32°C to 25°C by day 21. A 23 h light/1h dark cycle and a ∼30 to 40% relative humidity was maintained throughout the experiment. Birds were assigned to 1 of 6 dietary treatments in a complete randomized design: 1) a nutrient adequate positive control (**PC**) formulated to meet Cobb 500 nutrition requirements; 2) the PC diet with myo-inositol (**MI**, Sigma-Aldrich, St. Louis, MO) included at 0.3% (**PC + MI**); 3) a negative control (**NC**) with reduced available phosphorus, calcium, and sodium by 0.15, 0.16, or 0.03%, respectively; and 3 additional diets that used the NC treatment as a base in which QB (QB, AB Vista, Marlborough, UK) was added at either 500, 1,000, or 2,000 phytase units (**FTU**)/kg diet to create diets 4 (**NC + 500**), 5 (**NC + 1,000**), and 6 (**NC + 2,000**), respectively. Birds had *ad libitum* access to water and feed throughout the study.

Processing and Myopathy Scoring

Birds were processed on day 56 at the University of Arkansas Pilot Processing Plant (Fayetteville, AR) using a commercial inline system. Feed was removed from the birds 10 h before load out and processing, whereas *ad libitum* access to water remained constant. Live dock weights were recorded before being placed on a shackle line. Birds were electrically stunned, exsanguinated, soft scalded, de-feathered, and manually eviscerated. Breast fillets were subjectively hand scored for both woody breast and white striping (**WS**) on a scale of 1 increment with 0 being no signs of WB or WS, 1 was mild, 2 was considered moderate, and score 3 being severe WB or WS.

Fatty Acid Analysis

Fresh breast samples were collected from the cranial region on the pectoralis major ventral surface from unaffected and affected fillets. Samples were placed in a Labcono freeze-dryer (Labcono Corp., Kansas City, MO) and dried at −50°C for 72 h under a <10 mm of Hg vacuum. Dried breast samples were weighed (∼180 mg) into 30 mL tubes and subjected to direct transesterification by incubating in 0.2 mol methanolic KOH (2.0 mL) at 50°C for 45 min, with vortexing samples 2 to 3 times/min until dissolved (Murrieta et al., 2004). Once the samples cooled to room temperature, highly purified hexane (2 mL) and saturated aqueous NaCl (1 mL) were added to each tube. Tubes were then vortexed and centrifuged at 22°C for 5 min at 3,500 × *g* to suspend fatty acids. A 1.0 mm anhydrous sodium sulfate layer was placed in gas liquid chromatography (**GLC**) vials before transferring the fatty acid methyl esters. A GLC (model 5890 Series II GC with automatic sample injector [HP–7673] with HP–3365 software; Hewlett–Packard, Avondale, PA) was used to separate and analyze fatty acid methyl esters with a 60:1 split ratio.

Saturated fatty acid (**SFA**) total proportion was the weighted percentage sum of myristic (14:0), pentadecanoic (15:0), palmitic (16:0), margaric (17:0), and stearic (18:0) acids. Total proportions of monounsaturated fatty acids (**MUFA**) included myristoleic (14:1), palmitoleic (16:1*c*), 10-trans-heptadecenoic (17:1*t*), oleic (18:1*c*9), vaccenic (18:1*c*11), and gadoleic (20:1*c*11). Additionally, the polyunsaturated fatty acid (**PUFA**) total percent summed linoleic (18:2*n*6), γ-linolenic (18:3*n*6), α–linolenic (18:3*n*3), eicosadienoic (20:2), dihomo-γ-linolenic (20:3*n*6), arachidonic (20:4*n*6), eicosapentaenoic (20:5*n*3), docosapentaenoic (22:5*n*3), and docosahexaenoic (22:6*n*3). Total PUFA proportions were divided by total SFA proportions to determine PUFA:SFA. Total percent omega-3 fatty acids were calculated using α–linolenic (18:3*n*3), eicosapentaenoic (20:5*n*3), docosapentaenoic (22:5*n*3), and docosahexaenoic (22:6*n*3), whereas total omega-6 fatty acids

comprised linoleic (18:2*n*6), γ-linolenic (18:3*n*6), eicosadienoic (20:2), dihomo-γ-linolenic (20:3*n*6), and arachidonic (20:4*n*6).

Statistical Analyses

Data were analyzed using Student *t* test or one-way ANOVA when appropriate. Bird was the experimental unit, and score was considered an ordinal variable. The model included diet. When diet was significant, score means between diets were separated using Pearson Chi-square. Differences between the frequency of each score within diet was also determined using Fisher's exact test. If ANOVA revealed significant effects, the means were compared by Tukey multiple range test using the GraphPad Prism, version 6.0, for Windows (Graph Pad Software, La Jolla, CA), and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Since its characterization, WB continues to stymie the poultry industry (Sihov et al., 2014). Yet, its etiology is still not well defined. It is well known that the heavier the bird and the larger the quantity of relative meat, the more likely WB is to develop (Sandercock et al., 2009; Petracci and Cavani, 2012; Petracci et al., 2015). For example, breast scored for increased WB had increased BW (Table 1). Also, severe WB-affected birds exhibited a significantly higher hot and chilled carcass weight, wings, breast, tenders, leg, and rack weights compared with the unaffected group. Interestingly, these WB-affected birds had a significant lower abdominal fat percentage, but they manifested higher incidence of WS ($P < 0.01$) compared with unaffected broilers (Table 1). This indicates an increased breast muscle fiber fatty acid uptake in WB-affected birds which is supported by our previous study showing accumulation of intramyocellular lipids (de Almeida Mallmann et al., 2019) and an upregulation of muscle fatty acid translocase CD36 expression (data not shown). The increased intramyocellular lipid deposits in WB has been also observed in several previous studies (Velleman and Clark, 2015; Soglia et al., 2016). The mechanisms involved in this intracellular lipid accumulation and how these phenomena are involved in WB myopathy are relevant to understand WB etiology and merit further in depth investigations. It is possible that this fatty acid overload leads to oxidative stress and lipotoxicity (Holland et al., 2007; Verma et al., 2014).

Next, we sought, as a first step, to determine the fatty acid profiles in the breasts of healthy and severely WB-affected broilers. The GLC analysis showed that although the total percent of SFA did not differ between the 2 groups, myristic (14:0), pentadecanoic (15:0), and margaric (17:0) acid percent was higher ($P < 0.05$) in the breast of WB-affected birds compared with their unaffected counterparts (Table 2). Similarly, total MUFA percent was significantly higher in WB-affected compared with unaffected birds, with specific significant increases in myristoleic (14:1), palmitoleic (16:1c), 10-trans-heptadecenoic (17:1t), and oleic acid (18:1c9) (Table 2). However, vaccenic acid (18:1c11) was significantly lower in WB-affected compared with unaffected birds (Table 2). Gadoleic acid (20:1c11) levels did not differ between the 2 groups (Table 2). Interestingly, total PUFA and omega-3 percentages were significantly reduced in the breasts of WB-affected birds compared with their healthy counterparts (Table 2). Specifically, the percent of dihomo-γ-linolenic (20:3*n*6), arachidonic (20:4*n*6), eicosapentaenoic (20:5*n*3), docosapentaenoic (22:5*n*3), and docosahexaenoic (22:6*n*3) acid were lower ($P < 0.05$) in the breast of WB-affected compared with unaffected birds (Table 2). However, the levels of α- and γ-linolenic acids (18:3*n*3 and 18:3*n*6, respectively) were significantly higher in WB-affected compared with unaffected birds (Table 2).

Although it is still unknown at this time whether WB development is a consequence or a cause for fatty acid profile dysregulation, several lines of evidence showed a cytotoxic effect of SFA that leads to glucose

Table 1. Carcass parameters of WB-affected and unaffected broilers.

	Woody breast category[1]				
Item	NORM	MOD	SEV	SEM[2]	*P* value
Live weight, g	3,162.91[c]	3,657.88[b]	3,982.27[a]	57.96	<0.01
Hot carcass weight, g	2,368.23[c]	2,782.69[b]	3,096.77[a]	46.30	<0.01
Chilled carcass weight, g	2,416.66[c]	2,836.66[b]	3,149.92[a]	46.78	<0.01
Wings weight, g	247.56[c]	274.62[b]	297.24[a]	4.18	<0.01
Breast weight, g	605.80[c]	801.69[b]	992.61[a]	18.59	<0.01
Tenders weight, g	138.17[c]	162.80[b]	177.38[a]	3.58	<0.01
Leg weight, g	771.87[c]	867.65[b]	907.90[a]	15.15	<0.01
Rack weight, g	638.22[c]	715.98[b]	759.52[a]	11.97	<0.01
Fat, g	37.70	40.13	38.00	2.13	0.34
Fat, %	1.60[a]	1.47[a]	1.26[b]	0.09	<0.01
White striping[3]	0.78[c]	1.37[b]	2.42[a]	0.12	<0.01

[a-c]Means within a row without common superscript differ ($P \leq 0.05$).

[1]Woody breast score = 0, normal (NORM); 0.5 to 1.5, moderate (MOD); and 2 to 3, or severe (SEV).

[2]SEM = pooled SEM.

[3]White striping = a score from 1 to 3 with 1 being normal, 2 moderate, and 3 severe white striping.

Table 2. Fatty acid profile in the breast of WB-affected and unaffected broilers.

Item[3]	Woody breast score[1] 0	3	SEM[2]	*P* value
Saturated	32.61	32.14	0.21	0.13
14:0	0.45[b]	0.54[a]	0.01	<0.01
15:0	0.08[b]	0.10[a]	0.01	0.04
16:0	23.12	23.04	0.16	0.74
17:0	0.08[b]	0.12[a]	0.01	<0.01
18:0	8.88	8.34	0.23	0.11
Monounsaturated	38.52[b]	40.26[a]	0.59	0.05
14:1	0.08[b]	0.13[a]	0.01	<0.01
16:1*c*	4.25[b]	5.33[a]	0.17	<0.01
17:1*t*	0.02[b]	0.05[a]	0.01	0.01
18:1*c*9	0.14[b]	0.17[a]	0.01	0.05
18:1*c*11	3.85[a]	3.38[b]	0.01	<0.01
20:1*c*11	0.24	0.27	0.02	0.17
Polyunsaturated	26.17[a]	25.02[b]	0.39	0.05
18:2*n*6	17.80	18.14	0.21	0.30
18:3*n*6	0.13[b]	0.23[a]	0.02	<0.01
18:3*n*3	0.58[b]	0.69[a]	0.03	0.02
20:2	0.35	0.29	0.02	0.06
20:3*n*6	0.74[a]	0.61[b]	0.04	0.05
20:4*n*6	5.41[a]	4.35[b]	0.36	0.05
20:5*n*3	0.14[a]	0.07[b]	0.02	0.02
22:5*n*3	0.63[a]	0.43[b]	0.04	<0.01
22:6*n*3	0.39[a]	0.22[b]	0.03	<0.01
PUFA:SFA	0.80	0.78	0.01	0.19
Omega-3	1.73[a]	1.41[b]	0.06	<0.01
Omega-6	24.44	23.61	0.35	0.11

[a–b]Means with a row without common superscript differ ($P \leq 0.05$).

Abbreviations: PUFA, polyunsaturated fatty acids; SAF, saturated fatty acids.

[1]Woody breast score = 0, normal (NORM); 0.5 to 1.5, moderate (MOD); and 2 to 3, or severe (SEV).

[2]SEM = pooled SEM.

[3]Reported as a weight percentage.

dysmetabolism and mitochondrial function impairment associated with insulin resistance in mammalian skeletal muscle (Hirabara et al., 2010; Morales et al., 2017). Numerous studies in different tissues including muscle cell lines have shown that SFA may cause irreversible impairments of cellular functions by causing proteolysis and apoptosis (Staiger et al., 2006). This is likely the case in WB myopathy because several transcriptomic studies have suggested that glucose metabolism is dysregulated in the breast of WB-affected birds with an alteration in the glycolytic pathways (Mutryn et al., 2015). By using metabolomics approach, Abasht et al. suggested increased oxidative stress and accumulation of reactive oxygen species (**ROS**) in WB-affected broilers (Abasht et al., 2016). ROS are primarily generated in the electron transport chain of the mitochondria as byproducts of respiration (Turrens, 2003). A wide range of mitochondrial ROS-induced damage has been described, including mitochondrial DNA damage, protein carbonylation, and/or lipid peroxidation (Yakes and Van Houten, 1997: Costa et al., 2011). Mitochondrial DNA damage has been reported to induce mitochondrial dysfunction, apoptosis, and sarcopenia in mammalian skeletal muscle (Hiona et al., 2010). In a recent study, using transmission electron microscopy, we have clearly shown degraded mitochondria in disrupted muscle of WB-affected birds (de Almeida Mallmann et al., 2019). It is worth noting that because of limited literature in avian species and the newly emerged WB myopathy, we do not know if these fatty acids act individually, in combination, or if the (im)balance of SFA to PUFA ratio is most influential in the etiology and the pathogenesis of this metabolic disorder. For instance, omega-3 PUFA has been shown to improve mitochondrial function and prevent lipotoxicity and apoptosis (Schrauwen et al., 2010; Afshordel et al., 2015). Several studies suggested that omega-3 PUFAs prevent or reverse the impairment in skeletal muscle mitochondrial function by increasing fatty acid oxidation and mitochondrial biogenesis (Power and Newsholme, 1997; Lanza et al., 2013).

In a recent study, Greene et al. (2019) demonstrated that QB reduced the severity of WB via modulation of oxygen homeostasis-related pathways. Here, QB supplementation also reduces WS incidence in a dose-dependent manner (95.31, 75, and 58.62% incidence in NC+500, NC+1,000, and NC+2,000, respectively, compared with 89.74% in the control PC group). As abovementioned and in agreement with several previous studies, WB and WS are associated (the more WB is severe, the more likely WS is drastic) (Kuttappan et al., 2013; Sihvo et al., 2014; Soglia et al., 2016; Tijare et al., 2016). Quantum Blue has a high affinity for phytate and has been shown to influence serum nonesterified fatty acids and lipid metabolism in broilers (Liu et al., 2010) and modulate muscle fatty acid profile in shrimp (*Penaeus monodon*) (Biswas et al., 2007). The GLC analysis showed that QB superdosing reduced total SFA with specific decrease in stearic acid (18:0) in broilers (Table 3). Although total MUFA and PUFA contents were not changed with QB supplementation, the vaccenic acid (18:1c11) content was significantly decreased in the breast muscle of QB-fed birds (Table 3). This result is puzzling because vaccenic acid was also decreased in WB-affected birds. Interestingly, while the percentage of the dihomo-γ-linolenic (20:3n6) was decreased, QB supplementation increased most of the PUFA measured in this study, with a significant accretion in alpha-linolenic (18:3n3), gamma-linolenic (18:3n6), and eicosapentaenoic (**EPA**, 20:5n3) acids compared with the control diet (Table 3). The n-3 PUFA have been shown to reduce muscle wasting and increase the functional capacity of muscle by augmenting intracellular anabolic signaling (Smith et al., 2011; Smith et al., 2015). Several animal studies concluded that the omega-3 PUFA, that is alpha-linolenic and EPA, have positive effects on mitochondrial function in various mitochondrial dysfunction-related pathologies (Eckert et al., 2013). In addition to their anti-inflammatory properties (Vedin et al., 2008; Morin et al., 2017), n-3 PUFA ameliorate mitochondrial function by restoring the respiration rate, reducing ROS, and protecting the mitochondria against Ca^{2+}-evoked swelling and apoptosis (Taneda et al., 2010; Panasiuk et al., 2013). Omics, histological, and biochemical studies reported that WB is characterized by macrophage infiltration and exhibits higher amount of intracellular calcium (Sihvo et al., 2014; Mutryn et al., 2015).

Table 3. Effect of QB on fatty acid profile in broilers.

Item[1]	Diet						*P* value	*F(DFn, DFd)* *F(5, 30)*
	PC	PC + MI	NC	NC+ 500FTU	NC+ 1000FTU	NC+ 2000FTU		
SAF	33.74 ± 0.72	32.55 ± 0.54	32.49 ± 0.12	31.96 ± 0.53[2]	31.48 ± 0.12[2]	31.40 ± 0.40[2]	0.01	3.53
14:0	0.39 ± 0.02	0.42 ± 0.03	0.44 ± 0.03	0.50 ± 0.11	0.49 ± 0.2	0.48 ± 0.2	0.97	0.17
15:0	0.03 ± 0.03	0.06 ± 0.01	0.11 ± 0.01	0.09 ± 0.04	0.09 ± 0.02	0.08 ± 0.02	0.21	1.58
16:0	23.29 ± 0.68	22.74 ± 0.51	22.70 ± 0.45	22.97 ± 0.15	23.40 ± 0.23	23.57 ± 0.27	0.60	0.72
17:0	0.05 ± 0.02	0.08 ± 0.02	0.11 ± 0.01	0.10 ± 0.05	0.10 ± 0.03	0.09 ± 0.01	0.56	0.75
18:0	10.02 ± 0.31	9.26 ± 0.64	9.13 ± 0.48	8.29 ± 0.64	8.35 ± 0.26	8.18 ± 0.41[2]	<0.05	2.25
MUFA	36.99 ± 1.00	37.11 ± 2.04	36.71 ± 1.42	41.25 ± 1.98	38.44 ± 0.76	41.14 ± 1.09	0.10	2.04
14:1	0.04 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.11 ± 0.01	0.11 ± 0.08	0.12 ± 0.01	0.52	0.85
16:1*c*	3.88 ± 0.2	3.63 ± 0.47	3.72 ± 0.45	4.61 ± 0.54	4.61 ± 0.21	5.05 ± 0.39	0.08	2.14
17:1*t*	0.01 ± 0.009	0.02 ± 0.02	0.01 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.15	1.74
18:1*c*9	28.32 ± 0.92	29.05 ± 1.66	28.61 ± 1.08	32.49 ± 1.64	29.65 ± 0.66	32.05 ± 0.91	0.07	2.23
18:1t9	0.09 ± 0.02	0.12 ± 0.03	0.16 ± 0.01	0.18 ± 0.01	0.18 ± 0.006	0.19 ± 0.009	0.61	0.72
18:1*c*11	4.67 ± 0.22	4.01 ± 0.22	3.88 ± 0.23[2]	3.47 ± 0.19[2]	3.61 ± 0.16[2]	3.45 ± 0.13[2]	<0.01	5.57
20:1*c*11	0.12 ± 0.07	0.20 ± 0.05	0.26 ± 0.07	0.33 ± 0.04	0.26 ± 0.01	0.27 ± 0.01	0.08	2.19
PUFA	26.56 ± 0.71	27.56 ± 1.61	27.44 ± 1.07	24.45 ± 1.04	26.46 ± 0.57	24.19 ± 0.68	0.09	2.08
18:2*n*6	16.73 ± 0.17	18.51 ± 0.62	17.72 ± 0.52	17.91 ± 0.16	18.40 ± 0.50[2]	17.46 ± 0.10	0.05	2.50
18:3*n*3	0.37 ± 0.09	0.58 ± 0.07	0.57 ± 0.06	0.65 ± 0.04[2]	0.66 ± 0.06[2]	0.65 ± 0.04[2]	0.02	3.09
18:3*n*6	0.03 ± 0.01	0.13 ± 0.04	0.16 ± 0.04	0.10 ± 0.04	0.21 ± 0.04[2]	0.17 ± 0.04[2]	0.02	2.92
20:2	0.39 ± 0.1	0.37 ± 0.05	0.41 ± 0.05	0.31 ± 0.04	0.35 ± 0.03	0.26 ± 0.01	0.41	1.04
20:3*n*6	1.03 ± 0.09	0.75 ± 0.10	0.71 ± 0.12	0.56 ± 0.08[2]	0.70 ± 0.07[2]	0.63 ± 0.07[2]	0.01	3.50
20:4*n*6	6.87 ± 0.68	5.97 ± 1.28	6.31 ± 0.94	4.09 ± 1.01	4.98 ± 0.59	4.10 ± 0.59	0.15	1.70
20:5*n*3	0.06 ± 0.05	0.13 ± 0.04	0.20 ± 0.06	0.07 ± 0.05	0.23 ± 0.02[2]	0.16 ± 0.04	0.04	2.63
22:5*n*3	0.76 ± 0.07	0.70 ± 0.14	0.76 ± 0.10	0.46 ± 0.12	0.58 ± 0.07	0.47 ± 0.06	0.1	1.99
22:6*n*3	0.36 ± 0.1	0.42 ± 0.07	0.60 ± 0.13	0.29 ± 0.08	0.35 ± 0.05	0.29 ± 0.04	0.1	1.91
PUFA:SFA	0.79 ± 0.02	0.85 ± 0.05	0.84 ± 0.03	0.76 ± 0.01	0.82 ± 0.01	0.75 ± 0.01	0.04	2.54
Omega-3	1.54 ± 0.19	1.83 ± 0.14	2.13 ± 0.16[2]	1.47 ± 0.14	1.82 ± 0.08	1.57 ± 0.08	0.01	3.24
Omega-6	25.02 ± 0.69	25.73 ± 1.47	25.31 ± 0.93	22.98 ± 0.91	24.65 ± 0.51	22.62 ± 0.60	0.10	1.99

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAF, saturated fatty acids; PC + MI, PC supplemented with 0.3% myo-inositol; NC, negative control.

[1]Reported as a weight percentage.

[2]$P < 0.05$ compared with the control (PC).

Although the mechanism(s) by which QB modulate the fatty acid profile to ameliorate WB and WS is not known at this time point, it is possible that in addition to the improvement of mitochondrial function, n-3 PUFA promote a systemic environment that enhance satellite cell activity. An overall impairment in muscle regeneration in WB myopathy was recently demonstrated to be associated with alteration of satellite cell numbers and their ability to proliferate and differentiate (Clark and Velleman, 2016; Daughtry et al., 2017; Geiger et al., 2018). Saini et al. have shown that n-3 PUFA (EPA) promotes satellite cell differentiation via upregulating the expression of MyoD and myogenin (Saini et al., 2017).

In conclusion, this is the first report showing a shift in fatty acid profile in the breast of WB-affected birds and suggesting that QB-supplementation ameliorates WB and WS myopathy potentially via reducing SFA and increasing PUFA contents; however, further studies investigating the effect of individual or combination of PUFAs are warranted.

ACKNOWLEDGMENTS

This work was supported by grant from AB Vista (to SD). AB Vista had no role in conducting the research, generating the data, interpreting the results, or writing the manuscript.

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

Authors Mike Bedford and Carrie Walk are employed by company AB Vista. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic Perturbations in wooden breast disorder in chickens. PLoS One 11:e0153750.

Afshordel, S., S. Hagl, D. Werner, N. Rohner, D. Kogel, N. G. Bazan, and G. P. Eckert. 2015. Omega-3 polyunsaturated fatty acids improve mitochondrial dysfunction in brain aging–impact of Bcl-2 and NPD-1 like metabolites. Prostaglandins Leukot. Essent. Fatty Acids 92:23–31.

Biswas, P., A. K. Pal, N. P. Sahu, A. K. Reddy, A. K. Prusty, and S. Misra. 2007. Lysine and/or phytase supplementation in the diet of Penaeus monodon (Fabricius) juveniles: effect on growth, body composition and lipid profile. Aquaculture 265:253–260.

Bowker, B. 2016. Heavy Broilers Tough Problem: White Striping, Woody Breast. WATTAgNetePublishig Group, Rockford, IL. Accessed June 2020. https://www.wattagnet.com/articles/28645-chickens-tough-problems-white-striping-woody-breast?v=preview.

Clark, D. L., and S. G. Velleman. 2016. Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. Poult. Sci. 95:2930–2945.

Costa, R. A., C. D. Romagna, J. L. Pereira, and N. C. Souza-Pinto. 2011. The role of mitochondrial DNA damage in the citotoxicity of reactive oxygen species. J. Bioenerg. Biomembr. 43:25–29.

Daughtry, M. R., E. Berio, Z. Shen, E. J. R. Suess, N. Shah, A. E. Geiger, E. R. Berguson, R. A. Dalloul, M. E. Persia, H. Shi,

and D. E. Gerrard. 2017. Satellite cell-mediated breast muscle regeneration decreases with broiler size. Poult. Sci. 96:3457–3464.
de Almeida Mallmann, B., E. M. Martin, K. Soo Kim, N. L. Calderon-Apodaca, M. F. A. Baxter, J. D. Latorre, X. Hernandez-Velasco, L. Paasch-Martinez, C. M. Owens, S. Dridi, W. G. Bottje, E. S. Greene, and G. Tellez-Isaias. 2019. Evaluation of bone marrow adipose tissue and bone mineralization on broiler chickens affected by wooden breast myopathy. Front Physiol. 10:674.
de Brot, S., S. Perez, H. L. Shivaprasad, K. Baiker, L. Polledo, M. Clark, and L. Grau-Roma. 2016. Wooden breast lesions in broiler chickens in the UK. Vet. Rec. 178:141.
Eckert, G. P., U. Lipka, and W. E. Muller. 2013. Omega-3 fatty acids in neurodegenerative diseases: focus on mitochondria. Prostaglandins, Leukot. Essent. Fatty Acids 88:105–114.
Geiger, A. E., M. R. Daughtry, C. M. Gow, P. B. Siegel, H. Shi, and D. E. Gerrard. 2018. Long-term selection of chickens for body weight alters muscle satellite cell behaviors. Poult. Sci. 97:2557–2567.
Greene, E., S. Dadgar, B. Mallmann, S. Orlowski, A. Dhamad, S. Rochell, M. Kidd, C. Laurendon, H. Whitfield, C. Brearley, N. Rajaram, C. Walk, and S. Dridi. 2019. Quantum Blue reduces the severity of woody breast myopathy via modulation of oxygen homeostasis-related Genes in broiler chickens. Front Physiol. 10:1251.
Hiona, A., A. Sanz, G. C. Kujoth, R. Pamplona, A. Y. Seo, T. Hofer, S. Someya, T. Miyakawa, C. Nakayama, A. K. Samhan-Arias, S. Servais, J. L. Barger, M. Portero-Otin, M. Tanokura, T. A. Prolla, and C. Leeuwenburgh. 2010. Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. PLoS One 5:e11468.
Hirabara, S. M., R. Curi, and P. Maechler. 2010. Saturated fatty acid-induced insulin resistance is associated with mitochondrial dysfunction in skeletal muscle cells. J. Cell. Physiol. 222:187–194.
Holland, W. L., J. T. Brozinick, L. P. Wang, E. D. Hawkins, K. M. Sargent, Y. Liu, K. Narra, K. L. Hoehn, T. A. Knotts, A. Siesky, D. H. Nelson, S. K. Karathanasis, G. K. Fontenot, M. J. Birnbaum, and S. A. Summers. 2007. Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab. 5:167–179.
Hoppeler, H., and M. Vogt. 2001. Muscle tissue adaptations to hypoxia. J. Exp. Biol. 204:3133–3139.
Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724–2733.
Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013. Pathological changes associated with white striping in broiler breast muscles. Poult. Sci. 92:331–338.
Lanza, I. R., A. Blachnio-Zabielska, M. L. Johnson, J. M. Schimke, D. R. Jakaitis, N. K. Lebrasseur, M. D. Jensen, K. Sreekumaran Nair, and P. Zabielski. 2013. Influence of fish oil on skeletal muscle mitochondrial energetics and lipid metabolites during high-fat diet. Am. J. Physiol.. Endocrinol. Metab. 304:E1391–E1403.
Liu, N., Y. Ru, J. Wang, and T. Xu. 2010. Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. Br. J Nutr. 103:862–868.
Morales, P. E., J. L. Bucarey, and A. Espinosa. 2017. Muscle lipid metabolism: role of lipid Droplets and Perilipins. J. Diabetes Res. 2017:1789395.
Morin, C., L. Charbonneau, N. Ouellet, H. Ouellet, P. U. Blier, F. Dufresne, and S. Fortin. 2017. Eicosapentaenoic acid monoglyceride resolves inflammation in an ex vivo model of human peripheral blood mononuclear cell. Eur. J. Pharmacol. 807:205–211.
Mudalal, S., M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci. 2015. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal 9:728–734.
Murrieta, C. M., B. W. Hess, and D. C. Rule. 2004. Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Sci. 65:523–529.
Mutryn, M. F., E. M. Brannick, W. Fu, W. R. Lee, and B. Abasht. 2015. Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. BMC Genomics 16:399.
Panasiuk, O., A. Shysh, A. Bondarenko, and O. Moibenko. 2013. Omega-3 polyunsaturated fatty acid-enriched diet differentially protects two subpopulations of myocardial mitochondria against Ca(2+)-induced injury. Exp. Clin. Cardiol. 18:e60–e64.
Petracci, M., and C. Cavani. 2012. Muscle growth and poultry meat quality issues. Nutrients. 4:1–12.
Petracci, M., S. Mudalal, F. Soglia, and C. Cavani. 2015. Meat quality in fast-growing broiler chickens. World's Poult. Sci. 71:363–374.
Picchi, A. 2016. "Woody Breast" Could Bite the Chicken Business. CBS News. Accessed June 2020. https://www.wattagnet.com/articles/28645-chickens-tough-problems-white-striping-woody-breast?v=preview.
Power, G. W., and E. A. Newsholme. 1997. Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. J. Nutr. 127:2142–2150.
Saini, A., A. P. Sharples, N. Al-Shanti, and C. E. Stewart. 2017. Omega-3 fatty acid EPA improves regenerative capacity of mouse skeletal muscle cells exposed to saturated fat and inflammation. Biogerontology 18:109–129.
Sandercock, D. A., Z. E. Barker, M. A. Mitchell, and P. M. Hocking. 2009. Changes in muscle cell cation regulation and meat quality traits are associated with genetic selection for high body weight and meat yield in broiler chickens. Genet. Sel. Evol. 41:8.
Schrauwen, P., V. Schrauwen-Hinderling, J. Hoeks, and M. K. Hesselink. 2010. Mitochondrial dysfunction and lipotoxicity. Biochim. Biophys. Acta 1801:266–271.
Sihvo, H. K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.
Singh, P. K. 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: a review. World's Poult. Sci. J. 64:553–580.
Smith, G. I., P. Atherton, D. N. Reeds, B. S. Mohammed, D. Rankin, M. J. Rennie, and B. Mittendorfer. 2011. Omega-3 polyunsaturated fatty acids augment the muscle protein anabolic response to hyperinsulinaemia-hyperaminoacidaemia in healthy young and middle-aged men and women. Clin. Sci. 121:267–278.
Smith, G. I., S. Julliand, D. N. Reeds, D. R. Sinacore, S. Klein, and B. Mittendorfer. 2015. Fish oil-derived n-3 PUFA therapy increases muscle mass and function in healthy older adults. Am. J. Clin. Nutr. 102:115–122.
Soglia, F., S. Mudalal, E. Babini, M. Di Nunzio, M. Mazzoni, F. Sirri, C. Cavani, and M. Petracci. 2016. Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. Poult. Sci. 95:651–659.
Staiger, K., H. Staiger, C. Weigert, C. Haas, H. U. Haring, and M. Kellerer. 2006. Saturated, but not unsaturated, fatty acids induce apoptosis of human coronary artery endothelial cells via nuclear factor-kappaB activation. Diabetes 55:3121–3126.
Taneda, S., K. Honda, K. Tomidokoro, K. Uto, K. Nitta, and H. Oda. 2010. Eicosapentaenoic acid restores diabetic tubular injury through regulating oxidative stress and mitochondrial apoptosis. Am. J. Physiol. Ren. Physiol. 299:F1451–F1461.
Tijare, V. V., F. L. Yang, V. A. Kuttappan, C. Z. Alvarado, C. N. Coon, and C. M. Owens. 2016. Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. Poult. Sci. 95:2167–2173.
Turrens, J. F. 2003. Mitochondrial formation of reactive oxygen species. J. Physiol. 552:335–344.
Vedin, I., T. Cederholm, Y. Freund Levi, H. Basun, A. Garlind, G. Faxen Irving, M. E. Jonhagen, B. Vessby, L. O. Wahlund, and J. Palmblad. 2008. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. Am. J. Clin. Nutr. 87:1616–1622.

Velleman, S. G., and D. L. Clark. 2015. Histopathologic and myogenic gene expression changes associated with wooden breast in broiler breast muscles. Avian Dis. 59:410–418.

Verma, M. K., A. N. Yateesh, R. Smitha, K. Neelima, P. M. Pallavi, M. Reddy, J. Poornima, A. M. Oommen, M. R. Jagannath, and B. P. Somesh. 2014. Integrated analysis of chronic lipotoxicity on muscle metabolism and stress and its reversal by antioxidants. Springerplus 3:251.

Yakes, F. M., and B. Van Houten. 1997. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc. Natl. Acad. Sci. U. S. A. 94:514–519.