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Journal

Poultry Science, 98(1)

ISSN

0032-5791

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Publication Date

2019

DOI

10.3382/ps/pey327

Peer reviewed

The effects of an alternative diet regimen with natural methionine ingredients on white striping breast myopathy in broiler chickens

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ABSTRACT Conventional broiler diets include synthetic methionine to optimize fast muscle growth. Recently, a conventional synthetic methionine-rich diet was compared to alternative diet regimens providing natural sources of methionine. Broilers fed diets with natural methionine sources grew at a slightly slower rate. From this study, we hypothesized that the difference in a growth rate would be reflected in features of the breast muscle from broilers fed the alternative diet. We hypothesized that white striping of pectoralis major muscle would be reduced in slower growing broilers fed the alternative diet regimen with natural methionine. We also hypothesized that there would be associated differences in gene expression for cell differentiation and pathology markers. Broilers fed a conventional corn/soy diet regimen with synthetic methionine were compared to those fed roasted cowpea and sunflower seed meal (60% corn/soy, 20% sunflower seed meal, and 20% roasted cowpea) and no synthetic methionine. Overall broiler growth, muscle gene expression, and muscle collagen content data were compared. Expression analyses of combinations of *MYOD1*, *PPARG*, *COL1A2*, *TRIM63*, *SOD1*, *PTGS2*, and *CD36* genes were used to examine differentiation and inflammation in the pectoralis muscles. The group fed an alternative diet gained less weight than those fed the control diet in the starter and grower phases but not in the finisher phase. Ultimately, the conventional diet resulted in a greater final weight for the broilers. However, mean white striping scores for the pectoralis major muscles were greater in the conventional control diet regimen. Gene expression results indicated greater expression of *PPARG*, *PTGS2*, and *CD36* in the muscle of broilers fed the control diet. These data associate white striping with fat deposition and inflammation. Thus, whether due to differences in feed intake, growth rate, or actual compositional differences, the alternative diet with natural methionine sources seemed to curtail amounts of white striping in broiler muscle. More studies are necessary to further discern the effect of growth rate and natural methionine sources on white striping.

Key words: broiler, white striping, methionine, inflammation, fat deposition

2019 Poultry Science 98:413–421
http://dx.doi.org/10.3382/ps/pey327

INTRODUCTION

Broiler chickens are selected for and fed a diet to optimize the speed and quantity of muscle growth. The fast growth and high-energy diet, however, appears to compromise the quality of broiler muscle (Barbut et al., 2008; Petracci and Cavani, 2012). Although efficient growth is desirable for poultry producers, larger, fast-growing broiler chickens are more likely to display myopathies (Russo et al., 2015). As consumers determine which meat to buy based on aesthetic appearance, texture, and nutritional quality, it is in the industry's best interest to reduce such myopathies (Kuttappan et al., 2013). One myopathy associated with fast muscle growth is a fibrous white striping present across the breast muscle, which affects meat quality and could indicate pathophysiological issues with the muscle (Petracci et al., 2013; Russo et al., 2015; Sanchez Brambila et al., 2016; Soglia et al., 2016). Chicken breasts with white striping can result in more cook loss and can be characterized by regions with more fat, resulting in meat with a higher fat content and lower protein content that is also of poorer quality (Petracci and Cavani, 2012; Petracci et al., 2013). White striped breast muscle could also be affected by collagen content with increased collagen affecting the quality of meat (Petracci et al., 2013; Soglia et al., 2016).

When formulating broiler diets, it is important to provide nutrients efficiently. Broiler diets are developed with an emphasis on crude protein that is easily digestible into amino acids that can be reassembled and metabolized. Moreover, formulations are based on digestibility, economics, and ultimate quantity and quality of meat yield. Current corn- and soy-based diets are economical and promote accelerated growth.

Received November 13, 2017.
Accepted July 3, 2018.
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Efforts are underway to design a diet that will maintain desired growth rates while improving the quality of the meat. For example, vitamin E supplementation has been shown to decrease the incidence of PSE meat, supposedly by maintaining the integrity of cell membranes thus preventing the leakage of calcium ions and the stimulation of postmortem glycolysis (Zhang et al., 2011). Furthermore, methionine has been identified as the main limiting amino acid for broilers on a corn-soy diet (Si et al., 2004). Consequently, it has been shown that feeding a low-protein diet supplemented with methionine improves feed efficiency while minimizing feed costs compared to those on a typical diet or a low-protein methionine-deficient diet (Bunchasak and Keawarun, 2006). Added methionine fed to broilers has been offered through synthetic sources. Recent public interest has led some producers to consider using feed ingredients that are not synthetic, yet affordable and digestible. Therefore, the expectation is to create alternative diets using natural sources of methionine (Foster, 2017). To be beneficial for production, alternative diets will need to be safe, efficient, show comparable muscle growth to standard poultry diets, and meet consumer demands for quality and affordability. Naturally methionine-rich sources include fishmeal, sesame seed meal, and sunflower seed meal, for example (Jacob, 2013). In this study, cowpeas and sunflower seed meal were selected as alternative methionine sources because the by-products of these crops are readily available in California. Cowpeas are grown in California and Texas, and sunflowers are most popular in North Dakota but are also grown in Northern California (USDA, 2010). Cowpeas like black-eyed peas have been found to be comprised of 0.28 to 0.34% methionine (USDA, 2015). In this study, cowpeas were roasted because previous research demonstrated that the inclusion of this process reduced the concentration of trypsin inhibitors and thus increased the digestibility of amino acids like methionine (Taiwo et al., 1998).

In this study, as a follow-up study on alternative diet trials previously described (Foster, 2017), we investigated the breast muscle of broilers fed 1 of the 2 diet regimens, specifically comparing 1 diet rich in roasted cowpea and sunflower seed meal to that of a standard commercial diet. Broilers on the alternative diet regimen grew slightly slower than those fed a conventional diet (Foster, 2017). In this study, we hypothesized that the difference in a growth rate would be reflected in features of the breast muscle from broilers fed the alternative diet. We hypothesized that white striping of the pectoralis major muscle would be reduced in slower growing broilers fed a diet with natural methionine. We also hypothesized that there would be associated differences in gene expression for cell differentiation and pathology markers. To test this hypothesis, we examined growth rates resulting from each diet, examined pectoralis major muscle for white striping, assayed collagen content for the breast meat, and used gene expression to investigate differentiation status of cells in the pectoralis major, as well as pathophysiological mechanisms that might affect the pectoralis major and minor muscles in rapidly growing broilers.

MATERIALS AND METHODS

Growing Conditions and Weighing Birds

In total, 120 commercial Cobb 500 broilers were raised over the course of 6 wk, similarly to those grown under similar conditions at 30°C, with 12 birds per 1.22 m × 1.83 m pen, and given water *ad libitum*, according to an approved Institutional Animal Care and Use Committee protocol. The ratio of males to females was approximately 50:50. Sixty birds were fed a traditional 100% corn/soy diet, and sixty birds were fed a diet with by-products comprised of 60% corn/soy, 20% sunflower meal, and 20% roasted cowpea (Table 1). The diets were balanced for energy [metabolic energy value for each phase: 3,035 kcal/kg for starter (0 to 10 d), 3,108 kcal/kg for grower (11 to 22 d), and 3,180 kcal/kg for finisher phase (23 to 45 d)]. Total methionine content for control and alternative diets, respectively, were: 0.50 and 0.49% for the starter phase, 0.47 and 0.44% for the grower phase, and 0.46 and 0.47% for the finisher phase. Diet formulations were analyzed at the UC Davis Analytical Laboratory for nutrient content (Supplemental Table S1). Amino acid content for the diets was analyzed at the UC Davis Proteomics Core Facility (Supplemental Table S2). Weights of birds were monitored weekly and at the end of each phase using a bench scale. At day 0, the chicks were received as hatched that day.

White Striping Analysis

In total, 15 birds from each diet regimen, 3 from each pen randomly selected, were culled at day 45 using carbon dioxide. A gross analysis of the pectoralis major muscles was performed to determine the extent of white striping in birds fed the different diets. The extent of white striping was determined to be normal or mild (absent), moderate (observed but <1 mm thick), or severe (>1 mm thick), and scored 1, 2, or 3, respectively (Kuttappan et al., 2013). Outside of white striping, no significant pathology was noted for pectoralis major and pectoralis minor muscles.

Sample Collection and Sex Identification

Portions of the left pectoralis major and pectoralis minor muscles from the culled chickens were isolated, snap frozen in liquid nitrogen, and then stored at −80°C. Muscle tissue from each of the 30 birds was applied to produce genomic DNA lysate using the KAPA HotStart Mouse Genotyping Kit (KAPA Biosystems, Wilmington, Massachusetts). Polymerase chain reactions (PCR) were done using genotyping kit guidelines

Table 1. Diet ingredients.

	Starter (days 0 to 10) 298 g/bird	Grower (days 11 to 22) 1,011 g/bird	Finisher (days 23 to 45) 3,477 g/bird
	Traditional corn/Soy diet		
Ingredients	As fed (kg)	As fed (kg)	As fed (kg)
Organic corn, yellow	48.12	55.10	58.29
Organic soybean meal	46.39	39.33	35.86
Organic soybean oil	1.80	2.10	2.72
Dicalcium phosphate	1.67	1.49	1.38
Limestone, ground	1.03	1.07	0.91
Salt	0.45	0.40	0.38
DL-methionine 99%	0.26	0.23	0.19
Vitamin/Mineral mix–NRC	0.25	0.25	0.25
	Alternative diet		
Organic corn, yellow	0.00	0.61	9.41
Organic soybean meal	76.98	49.03	40.38
Organic cowpeas	8.00	20.00	20.00
Organic soybean oil	6.27	7.66	7.74
Organic sunflower meal	5.76	20.00	20.00
Dicalcium phosphate	1.31	0.98	0.88
Limestone, ground	1.03	1.05	0.92
Salt	0.45	0.40	0.38
Vitamin/Mineral mix–NRC	0.25	0.25	0.25

as well as a previously described sex identification protocol that relies upon an 18S rRNA control gene and the XhoI gene for a W-repeat-based-sexing strategy (Clinton et al., 2001). The distributions of female to male were 9:6 for the control group and 11:4, respectively.

Total RNA Isolation

Frozen samples were pulverized under liquid nitrogen, added to TRIzol Reagent, and then homogenized using a tissue homogenizer. Chloroform was added to the homogenate and the supernatant was collected after high-speed centrifugation at 4°C according to manufacturer's instructions (TRIzol, Invitrogen & ThermoFisher Scientific). For each sample, total RNA was isolated from the supernatant using the SurePrep TrueTotal RNA Purification Kit, according to manufacturer's instructions (Fisher BioReagents). Eluted total RNA for each sample was stored at −80°C. A NanoDrop microvolume UV spectrophotometer (ThermoFisher Scientific) was used to determine the concentrations and purities of the total RNA samples.

Reverse Transcription and Real-Time Quantitative PCR

The Applied Biosystems High Capacity cDNA Reverse Transcription Kit was used to reverse transcribe mRNA into single-stranded cDNA according to the manufacturer's instructions. For each sample, 1 μg of total RNA was added for reverse transcription (+RT), and 1 μg of total RNA was used as a negative control (−RT, no reverse transcriptase, thus no cDNA). Once reactions were complete, they were diluted for downstream use and stored at −20°C. Real-time quantitative PCR (RT-qPCR) was performed using the Applied Biosystems TaqMan Universal Master Mix II. The equivalent of 10 ng total RNA converted to cDNA was used for each reaction. Samples and TaqMan primer-probe sets were added to TaqMan Universal Master-Mix II (no UNG) according to manufacturer's instructions. For each 96-well reaction plate used, template cDNA and master mix/primer probe solution targeting the appropriate gene were added (+RT product in triplicate and −RT product in singlet). The RT-qPCR was performed using an Applied Biosystems StepOnePlus qPCR machine with StepOnePlus qPCR software, programmed according to the TaqMan Universal Master Mix II manual. Genes interrogated for pectoralis major and pectoralis minor muscle are presented in Table 2. Genes targeted for pectoralis major represented markers for cell differentiation and markers representing potential pathologies: *CD36* (foam cell marker), *COL1A2* (type I collagen), *MYOD1* (muscle differentiation), *PPARG* (adipose differentiation), *PTGS2* (inflammation marker), *SOD1* (oxidative stress marker), and *TRIM63* (muscle atrophy marker). To examine pathology at the molecular level for pectoralis minor, gene expression for *PTGS2, SOD1*, and *TRIM63* were assayed.

Table 2. Gene targets for RT-qPCR.

Gene Symbol	Applied Biosystems TaqMan ID	Exons targeted
RER1	Gg03369124_m1	3 and 4
CD36	Gg03354937_m1	8 and 9
COL1A2	Gg03325897_m1	51 and 52
MYOD1	AIKAMLZ	2 and 3
PPARG	Gg03345639_m1	4 and 5
PTGS2	Gg03320008_m1	7 and 8
SOD1	Gg03348481_m1	2 and 3
TRIM63	Gg03310773_m1	2 and 3

Hydroxyproline Collagen Assay

The hydroxyproline collagen assay quantifies the amount and percent of collagen in each sample. Approximately, 0.3 g of wet mass samples of pectoralis major tissue were dried in an oven at 120°C for 20 to 25 min. Roughly, 0.04 g of dried sample was weighed out for further collagen analysis. Samples were incubated in 6 N HCl on a heat block at 120°C for 12 h in pressure tight glass vials. The vials were cooled slightly, spun down to collect condensate, and replaced on the heating block without caps to evaporate for 4 h at 120°C. The sample pellet was then re-suspended in 200 μL of hydroxyproline buffer. Standards were made using trans-4-hydroxy-L-proline (Sigma-Aldrich) at concentrations of 0, 5, 10, 15, 20, 30, 40, and 80 μg/mL in hydroxyproline buffer, and 200 μL aliquots of each were made. A 1:4 dilution of 40 μL sample and 160 μL buffer and was used to analyze the samples; for each sample and standard, 150 μL of 14.1 mg/mL chloramine-T solution was added and samples were incubated at room temperature for 20 min. Subsequently, 150 μL of aldehyde-perchlorate solution was made fresh and added to the standards and samples, vortexed, and incubated at 60°C (Woessner, 1961). Samples and standards were pipetted into a 96-well flat-bottom plate in triplicate and read with a UV/Vis absorbance microplate reader measuring absorbance at 550 nm (Woessner, 1961; Edwards and O'Brien, 1980; Dunkman et al., 2014).

Data Analysis

RT-qPCR data were transferred from the StepOnePlus software to Microsoft Excel and processed so that the amplification curves could be analyzed in LinReg software (Ramakers et al., 2003). The best fit line between 4 and 6 points with an $R^2 > 0.99$ was used to determine efficiency (eff). Mean efficiencies and their standard deviations were found for each group on each plate to discern whether or not statistically significant differences were present (unpaired *t*-test) (Mienaltowski et al., 2008; Mienaltowski et al., 2009). With no significance detected, mean efficiency of each gene for each plate was used (Ramakers et al., 2003; Schefe et al., 2006; Mienaltowski et al., 2008; Mienaltowski et al., 2009). Relative expression (**RE**) was found for each replicate as previously described (Mienaltowski et al., 2008). Housekeeping gene *RER1* (*retention in endoplasmic reticulum 1*) was used for normalization. Data were entered into and analyzed within GraphPad Prism software to find means, and applied to two-way ANOVA tests by diet and sex (Table 3). Significance was defined as $P \leq 0.05$.

Analysis of data for the hydroxyproline collagen assay was performed similarly. Excel was used to plot a best-fit line of absorbance vs. mass from the measurements of the hydroxyproline standards. Micrograms of collagen per milligrams tissue and percent dry mass of collagen were calculated for each sample. Means per group and a two-way ANOVA were then calculated in GraphPad Prism (Table 3).

Table 3. Report of statistical analyses for RT-qPCR and hydroxyproline assays.

	Diet	Sex	Interaction
	Pectoralis major		
COL1A2 expression	0.44	0.68	0.092
MYOD expression	0.85	0.82	0.025
PPARG expression	0.010	0.90	0.80
TRIM63 expression	0.49	0.71	0.49
PTGS2 expression	0.035	0.74	0.32
CD36 expression	0.0275	0.87	0.48
SOD1 expression	0.42	0.91	0.030
[Collagen] dry mass %	0.95	0.85	0.78
Collagen content (μg/mg)	0.84	0.73	0.47
	Pectoralis minor		
TRIM63 expression	0.031	0.086	0.0032
PTGS2 expression	0.42	0.71	0.55
SOD1 expression	0.38	0.26	0.70

RESULTS

Production Performance

In total, 57 of the birds fed the traditional diet and 54 of the birds fed the alternative diet survived to market weight; weights of the surviving birds were monitored weekly and at the end of each phase. There was a significant difference in weights between groups in the starter, grower, and finisher phases of production ($P < 0.01$) (Figure 1A and B). However, there was only a significant difference in weight gain during the starter and grower phases (Figure 1C). The group fed an alternative diet regimen gained less weight than the control in the starter and grower phase ($P < 0.01$), but not in the finisher phase ($P = 0.49$) (Figure 1C).

White Striping Scores

Upon performing a gross visual analysis after the broilers were culled, the alternative group had an increase in mild white striping and a decrease in severe white striping compared to the group fed a control diet, with mean white striping scores of 2.1 and 2.6, respectively ($P = 0.068$) (Figure 2) with no significant contribution of sex ($P = 0.56$).

Pectoralis Major Gene Expression and Collagen Content

There was a significant difference in the RE of *PPARG* ($P = 0.010$) in the pectoralis major muscles between control and alternative diet regimen groups, with no statistical difference found for the *MYOD* and *COL1A2* groups (Figure 3). Gene expression for *PPARG* was 2.50× greater in the control diet. This indicates that there was less differentiation of adipose tissue in the alternative diet than the control diet, but no difference in the differentiation of muscle tissue or

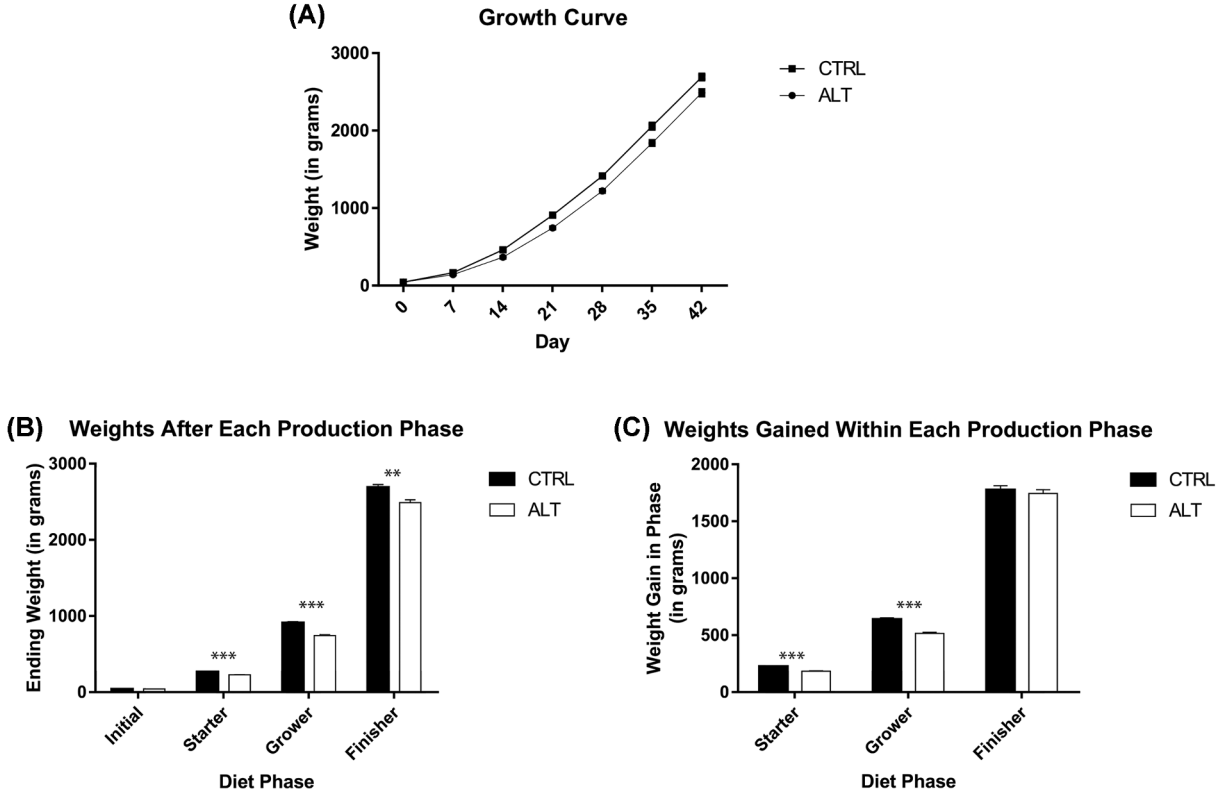

Figure 1. Comparing the growth of broilers fed control and alternative diet regimens. Graphs display weight at each week **(A)**, weights after each production phase **(B)**, and weight gained within each production phase **(C)**. Mean ± SEM are given. Significance denoted by an asterisk: * ($P < 0.05$), ** ($P < .01$), *** ($P < 0.001$).

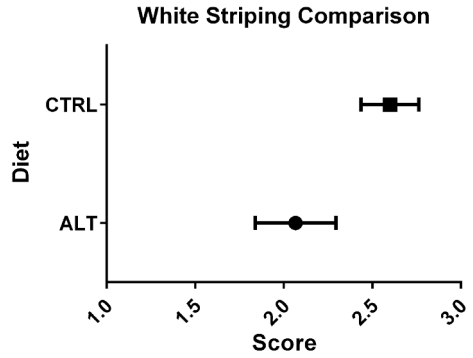

Figure 2. Comparison of white striping between broilers fed alternative and control diet regimens. Results of a gross analysis of pectoralis major and pectoralis minor muscles in broilers fed with control (CTRL) and alternative (ALT) diets. Distributions are plotted as mean ± SEM, $P = 0.068$.

collagen. There was also a significant difference in RE of *CD36* ($P = 0.028$) and *PTGS2* ($P = 0.035$) between the control and the alternative diet regimen groups. Gene expression for foam cell marker *CD36* and inflammation marker *PTGS2* were 1.66× and 2.04× greater, respectively, in the control diet regimen. No significant difference was found for *SOD1* ($P = 0.42$) and *TRIM63* ($P = 0.49$). No sex-dependent differences were found for any of the genes (Table 3). According to the hydroxyproline collagen assay, there was no difference in the percent dry mass of collagen (Figure 4A) or μg collagen per mg tissue (Figure 4B) by diet or sex (Table 3).

Pectoralis Minor Gene Expression

The pectoralis minor muscles showed no statistically significant difference in the expression of the *SOD1* or *PTGS2* genes by diet or sex, suggesting no difference in inflammatory processes through their respective pathways (Figure 5). The pectoralis minor muscles did have a diet-dependent difference in expression for *TRIM63* (Table 3) ($P = 0.031$).

DISCUSSION

As the broiler industry focuses on larger, faster-growing birds, meat quality is being compromised for meat quantity. In order to examine if an alternative diet might affect the incidence of myopathies, particularly white striping, while allowing for comparable growth rates, this study compared broilers fed a traditional corn/soy diet regimen with synthetic DL-methionine to an alternative diet regimen containing corn/soy, cowpeas, and sunflower seed meal. The latter diet contained no synthetic methionine. Upon comparing the growth curves of the 2 groups to the incidence of white striping, it appears that the faster growing birds tended to have more white striping.

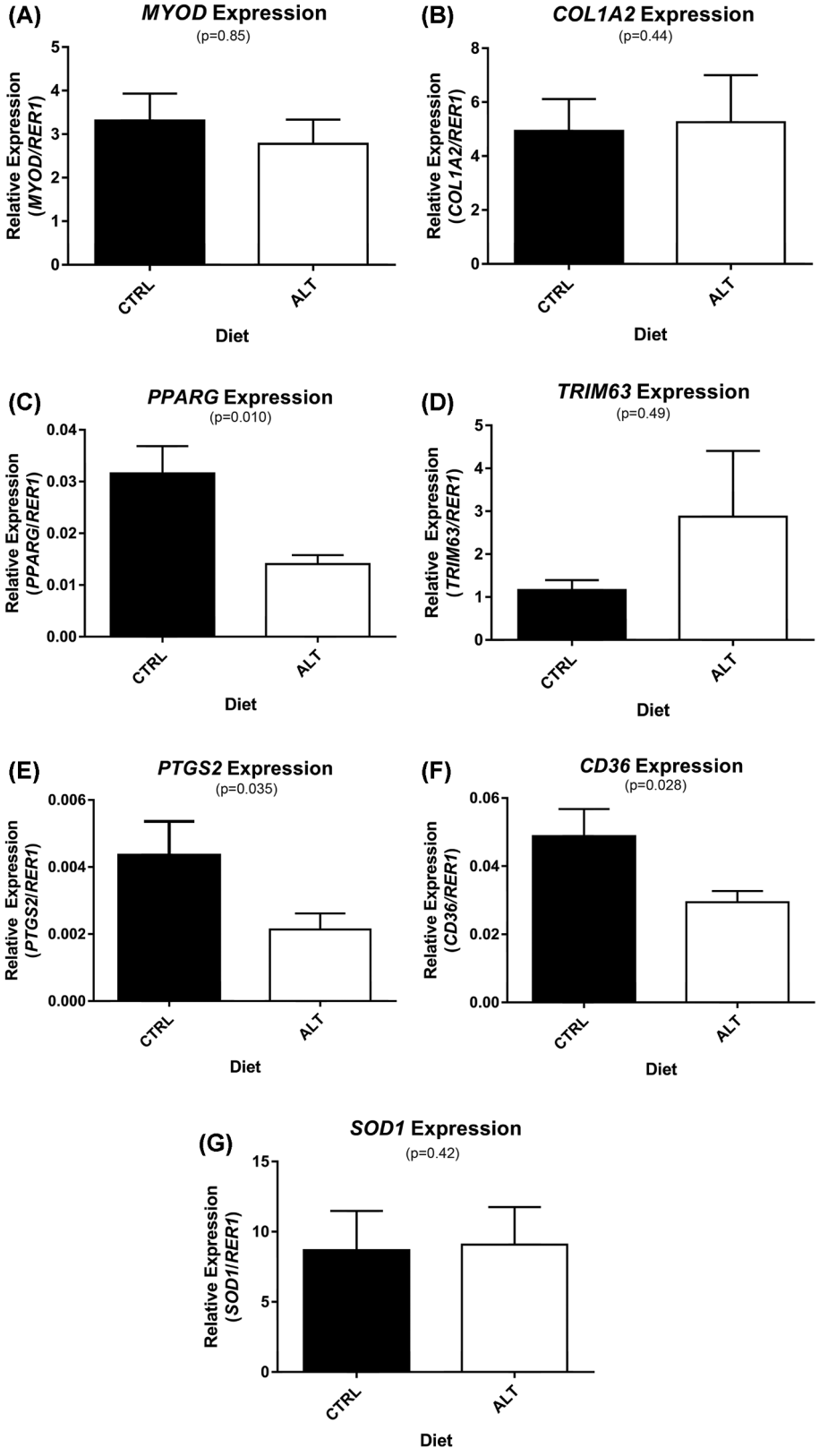

Figure 3. Expression of differentiation and physiology markers in pectoralis major muscles. Gene expression was measured by RT-qPCR with results given as mean ± SEM. Transcript abundance for **(A)** *MYOD*, **(B)** *COL1A2*, **(C)** *PPARG*, **(D)** *TRIM63*, **(E)** *PTGS2*, **(F)** *CD36*, and **(G)** *SOD1* genes in pectoralis major muscles for the control (CTRL) and alternative (ALT) diet regimen groups, relative to housekeeping gene *RER1*.

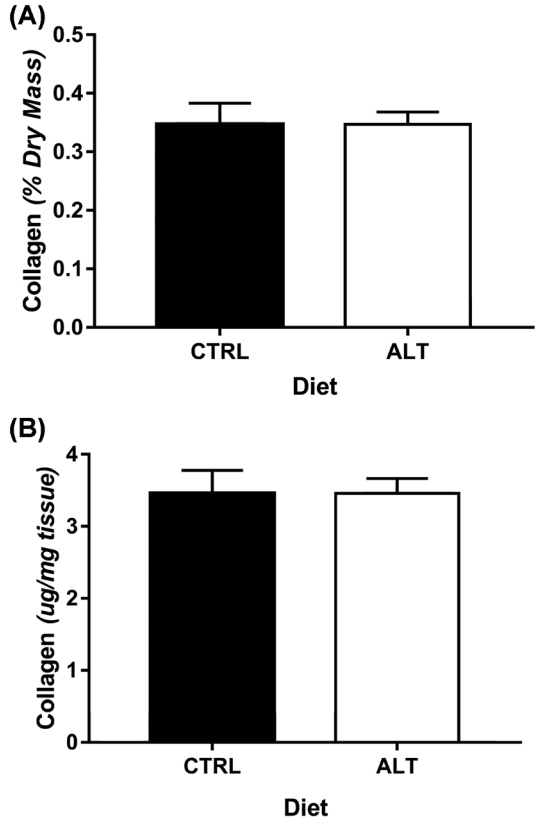

Figure 4. Analysis of the collagen content in the pectoralis major muscles of broilers fed a control (CTRL) or alternative (ALT) diet regimen. Collagen content for both **(A)** percent concentration of collagen in the dry mass in the tissues and **(B)** collagen content relative to wet weight, with results given as mean ± SEM. No significant differences found.

One interesting difference was found based upon the differentiation status. There was increased expression of *PPARG*—a gene that regulates fatty acid storage, glucose metabolism, lipid uptake, and adipogenesis—in the control diet for pectoralis major muscle. This suggests that the commercial diet regimen resulted in increased fat deposits within the muscle. Previous work has suggested that fat deposition contributes to the significance of white striping (Petracci and Cavani, 2012; Petracci et al., 2013; Soglia et al., 2016). It is possible that the difference in white striping seen between the 2 diets is a result of inefficiencies in the alternative diet regimen that thus slowed broiler growth and fat deposition. The alternative diet regimen studied here did have decreased intake and digestibility when compared to the control commercial diet regimen (Foster, 2017). A notable lack of expression difference for *MYOD1* is suggestive that the increased growth of the control group is not due to an increase in muscle. However, for broilers fed the alternative diet, expression of *TRIM63*—a marker also known as Muscle RING-finger protein-1 or MuRF1 that is essential in the control of muscle atrophy—was upregulated in the pectoralis minor and was trending upward in pectoralis major muscle. Regulation of muscle protein synthesis involves TRIM63, or MuRF1, particularly with changes in energy metabolism (Koyama et al., 2008; Tesseraud et al., 2009; Li et al., 2011; Bodine and Baehr, 2014). Upregulation of *TRIM63* is generally associated with a loss of muscle mass, particularly in cases of starvation or nutritional deficiencies (Li et al., 2011; Bodine and Baehr, 2014), which could provide an explanation as to a mechanism by which there is less growth in the broilers fed an alternative diet, particularly when compared to growth rates of the conventional control diet. In future studies, it would be beneficial to measure in each group the total fat content of the muscles as well as muscle mass to determine if and to what extent the higher weights of the control group compared to the alternative group is due to an increase in fat and/or muscle mass.

For the birds of this study, it has been determined that differences between groups are not due to differences in collagen content. As it was determined that the expression of *COL1A2* was not different between groups at the time of harvesting based on the real-time qPCR results and the hydroxyproline collagen assay, respectively, it would be useful to determine if the expression of *PPARG* and *MYOD1* varied at different stages of growth. This could potentially explain how it would be possible for control birds to have greater muscle mass than the alternative group if they express more *MYOD1* earlier in development, and then subsequently use the energy from the diet more for building adipose tissue in later stages. This could be particularly useful information since differences in final weights between groups appear to be due to differences in early growth. Future studies will examine muscle differences due to diet at several time points through growth and not just market weight.

The increased expression of foam cell marker CD36 should be noted. Increased expression of the *CD36* gene, which encodes fatty acid translocase, was seen in the pectoralis major muscle of the control diet regimen. Fatty acid translocase incorporates lipids into cells. When increased expression of CD36 is coupled with increased expression of inflammatory marker *PTGS2* in the pectorals major muscles of broilers fed the control diet regimen, it is plausible to consider that the combination of fat deposition and inflammatory conditions could lead to increased levels macrophages taking up lipids, resulting in foam cells, particularly as a part of atherogenesis (Shashkin et al., 2005). Peptides from roasted cowpeas have been shown to have antioxidant activity, to inhibit cholesterol synthesis, and to prevent cholesterol solubilization into micelles (Marques et al., 2015). These properties are considered anti-atherosclerotic in humans; in the broiler, roasted cowpeas could be preventing foam cell formation by virtue of these activities. Future studies of this tissue will include specific analysis of these cells in broiler muscle and mechanisms by which roasted cowpeas in the diet could contribute to decreases in white striping.

A significant limitation of this study is that the diet regimens differed in multiple nutrients other than

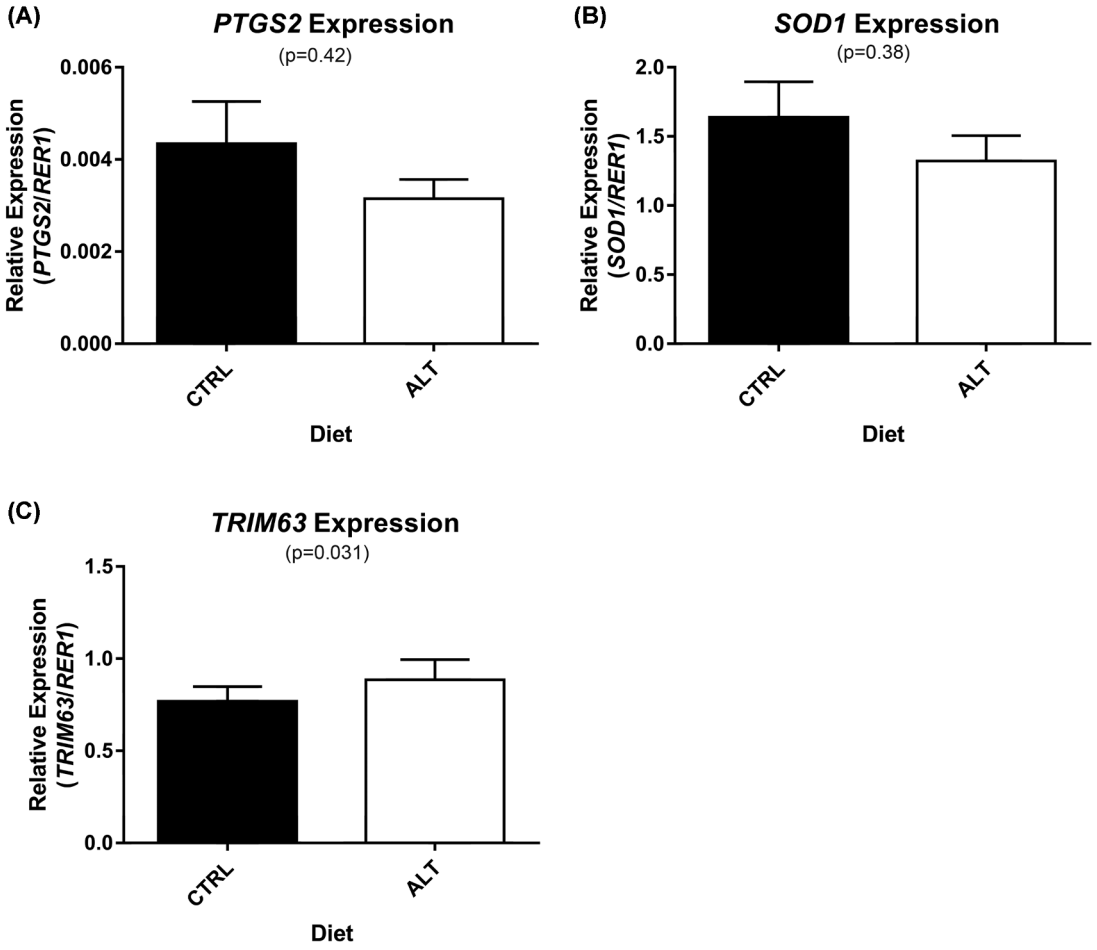

Figure 5. Expression of differentiation markers in pectoralis minor muscles. RT-qPCR results for **(A)** *PTGS2*, **(B)** *SOD1*, and **(C)** *TRIM63* genes, relative to housekeeping gene *RER1*, in pectoralis major muscles for the control (CTRL) and alternative (ALT) diet groups. Values given as mean ± SEM.

methionine. As the diets were balanced against each other only for their energy and methionine content, it is likely that the different diet regimens do not necessarily compare how different sources of methionine affect growth and white striping but instead how nutrient availability affects growth and white striping. That is, the alternative diet regimen contained more protein, acid detergent fiber, cellulose, and crude fat than the control diet (See Supplemental Tables S1 and S2) and formulations were close to those recommended by the Cobb-Vantress, but its intake was less relative to the control diet (Foster, 2017). As a result, it would be useful to know which nutrients in which combinations have a positive effect on muscle growth and health. However, the control diet regimen had a higher percent of total digestible nutrients, which could indicate that the broilers were not absorbing certain nutrients in the alternative diet as efficiently as they might otherwise have, perhaps due to the significantly higher fiber content. Nonetheless, the lessened incidence of white striping may suggest that in some ways the nutrition obtained from the alternative diet was superior to that obtained from the control diet, though producers would still need to weigh any concerns with affordability and management. Thus, although the birds fed a control diet regimen grew larger, this slightly increased quantity of meat contrasts with the higher quality of meat in those birds fed an alternative diet regimen containing cowpeas and sunflower seed meal at least as far as incidence of white striping. As the alternative group had lesser RE of *PPARG* in the pectoralis major muscles, such a diet may be beneficial to the production of leaner breast meat that is desirable to consumers, though producers would need to consider management and profitability associated with changes. Likewise, with potential benefits of roasted cowpeas documented in the human nutrition literature, the associated decreased levels of *CD36* expression and white striping provide compelling arguments to further study the effects of these methionine-rich alternatives in the broiler diet.

Thus, in conclusion, although the birds fed a control diet grew larger, this slightly increased quantity of meat contrasts with the higher quality of meat in those birds fed an alternative diet containing cowpeas and sunflower seed meal due to the decreased incidence of white striping. The breast muscle of the control diet regimen broilers had increased expression of *PPARG*, *PTGS2*, and *CD36*, which are markers for

fat deposition, inflammation, and foam cell formation, respectively. Differential expression of a foam cell marker adds insight into a potentially new mechanism to consider when managing white striping in production, particularly when atherogenic mechanisms are reduced when roasted cowpeas are included in human nutrition. From this study, we affirm that coupling the current highly efficient corn/soy (control) diet with the genetics of today's broilers may improve growth rates but can also introduce mechanisms that can adversely affect breast muscle quality. Future studies will include further discerning how roasted cowpeas and sunflower seed meal affect broiler growth and slow or prevent striping.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

Supplemental Tables 1. Nutrient compositions for control and alternative diets.
Supplemental Table S2. Amino Acid Composition of Diets, by percent.

ACKNOWLEDGMENTS

We gratefully acknowledge support from the Department of Animal Science, College of Agricultural and Environmental Sciences, and the California Agricultural Experiment Station of the University of California-Davis for funding the research affiliated with USDA Multi-State Project NC 1184: Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation. We thank Foster Farms for the donation of the broiler chicks and commercial diet ingredients. We are also grateful to Associated Feed for donation of organic ingredients.

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