

Research Note

Effect of the timing of posthatch feed restrictions on the deposition of fat during broiler breast muscle development¹

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ABSTRACT The effect of the timing of posthatch feed restriction on adipose deposition and adipogenic gene expression in the broiler pectoralis major muscle was studied by applying a 20% feed restriction either the first or second week after hatch. Broiler chicks at hatch were divided into a full-fed (control) group and a 20% feed restriction group. The expression of adipogenic genes, peroxisome proliferator-activated receptor gamma (PPAR γ), and CCAAT/enhancer-binding protein alpha (C/EBP α) were measured. The expression of both PPAR γ and C/EBP α was affected by the wk 1 feed restriction with expression significantly increased

during the first week posthatch. The deposition of fat within the pectoralis major muscle was affected by the timing of the feed restriction. Extensive fat depots were present by 27 d of age in the pectoralis major muscle of the wk 1 restricted group compared with the control. Fat deposition was eliminated when the 20% feed restriction occurred in wk 2. Taken together, these results demonstrate that the timing of early posthatch feed restrictions in chicks is critical in the deposition of fat in the pectoralis major muscle and expression of adipogenic genes.

Key words: chick, feed restriction, fat, adipogenic gene, muscle

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INTRODUCTION

Posthatch muscle growth through the process of hypertrophy is mediated by the adult myoblast stem cell population termed satellite cells. The satellite cells were first identified by Mauro (1961) as being located between the basement membrane and sarcolemma of skeletal muscle fibers. Muscle growth through hypertrophy occurs by the satellite cells fusing with and donating their nuclei to existing muscle fibers (Stockdale and Holtzer, 1961; Moss and LeBlond, 1971), increasing protein synthesis potential.

The first week posthatch in broilers has been shown to be the period of maximal satellite cell activity, and satellite cells are sensitive to nutritional regimen during this time (Halevy et al., 2000). Satellite cells are a multipotential mesenchymal stem cell population with plasticity to commit to myogenesis or alternative differentiation programs such as osteogenesis or adipogenesis (Asakura et al., 2001; Shefer et al., 2004). During

the period of maximal satellite cell activity, the satellite cells are sensitive to nutrition. Halevy et al. (2000) showed that the timing of a 2-d feed deprivation affected muscle mass accretion. The closer the 2-d feed deprivation was to hatch, the greater the reduction in the ability of the chick to have compensatory growth. Velleman et al. (2014) using a 20% feed restriction during the first or second week after hatch showed that restricting feed during the first week posthatch altered the morphological structure of the pectoralis major muscle and the expression of key myogenic genes. In contrast, administering the feed restriction during the second week posthatch eliminated the morphological changes in the pectoralis major muscle and differences in gene expression. In support of the findings of Velleman et al. (2014), Plavnik and Hurwitz (1988, 1990) showed that restricting feed the second week after hatch resulted in complete compensatory growth. Thus, muscle growth, pectoralis major morphological structure, and the expression of key myogenic genes are affected by the timing of a posthatch feed restriction. Because the satellite cells are multipotential stem cells, the objective of the present study was to determine the effect of timing of a posthatch feed restriction on the deposition of fat within the pectoralis major muscle and the expression of the adipogenic transcription factors peroxisome proliferator-activated receptor gamma (PPAR γ) and CCAAT/enhancer binding protein α (C/EBP α).

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The expression of these adipogenic transcription factors in myoblasts has been shown to activate the transdifferentiation of myoblasts to an adipogenic lineage (Hu et al., 1995; Yu et al., 2006).

MATERIALS AND METHODS

Birds

Fertile eggs from the Ross 308 broiler line were shipped to The Ohio Agricultural Research and Development Center Chicken Research Facility of The Ohio State University (Wooster). In 2 trials, males were raised with 240 birds per trial (Velleman et al., 2014). For the wk 1 feed restriction treatment, a 20% feed restriction was administered from hatch (0 d) through 7 d of age and after 7 d the chicks were fed ad libitum. At d 0, the chicks were divided into a control group on a standard full-fed diet and a 20% feed-restricted group. After the separation into experimental groups, 8 birds from each group were humanely killed with BW and pectoralis major muscle weight recorded, a sample was removed for histological analysis, and a sample was removed for RNA analysis and placed in RNAlater (Ambion, Austin, TX). Every day through 7 d of age, all the birds from both groups were weighed. In addition to the BW, 8 birds from each group were killed, pectoralis major muscle weight was recorded, and samples removed for RNA analysis and histological analysis. After 7 d of age, the birds were weighed and sampled as described every 4 d through 43 d of age. In the wk 2 feed restriction treatment, which was done independent of the wk 1 feed restriction experiment, all the chicks were full-fed from d 0 through d 7, and on 8 d of age through 14 d the feed-restricted group was administered a 20% feed restriction, and the control birds were fed ad libitum. In both trials, the 20% feed restriction was a reduction in feed mass and was accomplished by measuring the amount of feed consumed by the control group on a daily basis during the period of the feed restriction and adjusting the feed allocation of the restricted birds. After the restriction, all birds were fed ad libitum. At 0 d, the chicks were divided into a control full-fed group and a 20% feed restriction group. After the separation into experimental groups, 8 birds from each group were killed, and BW and pectoralis major muscle weight were recorded; a sample was removed for histological analysis, and a sample removed for RNA analysis and placed in RNAlater. Every 2 d through 14 d of age, all the birds from both groups were weighed and BW recorded. Eight birds from each group were killed, pectoralis major muscle weight recorded, and samples removed for RNA analysis and histological analysis. After 14 d of age, the birds were weighed and sampled every 4 d through 42 d of age. Body weights and pectoralis major muscle weights for the wk 1 and 2 feed restrictions were reported previously in Velleman et al. (2014).

Histology and Morphometric Analysis

After the removal of the skin from the breast region, a sample of the breast muscle was obtained by carefully dissecting approximately a 0.3 to 0.5 cm wide section of the muscle following the orientation of the muscle fibers for a length of about 3 cm. Samples were processed and embedded as described in Jarrold et al. (1999). The resulting paraffin blocks were cross-sectioned at 5 μ m and mounted on Starfrost Adhesive slides (Mercedes Medical, Sarasota, FL). Hematoxylin and eosin staining was done as described in Velleman and Nestor (2004). The stained muscle sections were analyzed for muscle morphology and fat deposition with an Olympus XI 70 microscope (Olympus America, Melville, KY) and a QImaging digital camera (QImaging, Burnaby, BC, Canada) with CellSens software (Olympus America, Melville, KY). Sections from each bird were rated by 4 individuals for fat deposition. The ratings ranged from 1 (no observable fat deposition) to 5 (extensive fat deposition).

Total RNA Extraction and cDNA Synthesis

Pectoralis major tissue was collected at hatch through 42 or 43 d of age and stored in RNAlater until being extracted using RNazol RT (Molecular Research Center, Cincinnati, OH) according to the manufacturer's protocol. The RNA was pooled from 5 birds per treatment and age, and the cDNA were synthesized as described in Velleman et al. (2014).

Real-Time Quantitative PCR

Real-time quantitative PCR (qPCR) was performed using the DyNAmo Hot Start SYBR Green qPCR kit (ThermoFisher, Pittsburgh, PA) with a DNA Engine Opticon 2 real-time system (Bio-Rad, Hercules, CA). Each real-time qPCR reaction consisted of 2 μ L of cDNA, 10 μ L of 2 \times master mix, and 1 μ L of 10 μ M primer mixture (forward and reverse) of the target genes PPAR γ , C/EBP α , or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 7.0 μ L of nuclease-free water for a 20- μ L reaction volume. The PPAR γ primer sequences were (GenBank accession number NM_001001460.1) forward primer 5'-₈₀₅CCACTGCAGGAACAGAACAA₈₂₄-3' and reverse primer 5'-₁₀₅₄CTCCCGTGTCATGAATCCTT₁₀₃₅-3', and C/EBP α (GenBank accession number NM_001031459.1) forward primer 5'-₇₂₈CAGTGGACAAGAACAGCAACGA₇₄₉-3' and reverse primer 5'-₉₅₅CCTTAC-CAGCGAGCTTTTCG₉₃₆-3'. The GAPDH primer sequences (GenBank accession number U94327) were forward primer 5'-₅₀₄GAGGGTAGTGAAGGCTGCTG₅₂₃-3' and reverse primer 5'-₇₀₃CCACAACACGGTTGCTGTAT₆₈₄-3'. The specificity of each of these gene-specific primers was confirmed by DNA sequencing of the amplified sequence product (Molecular and

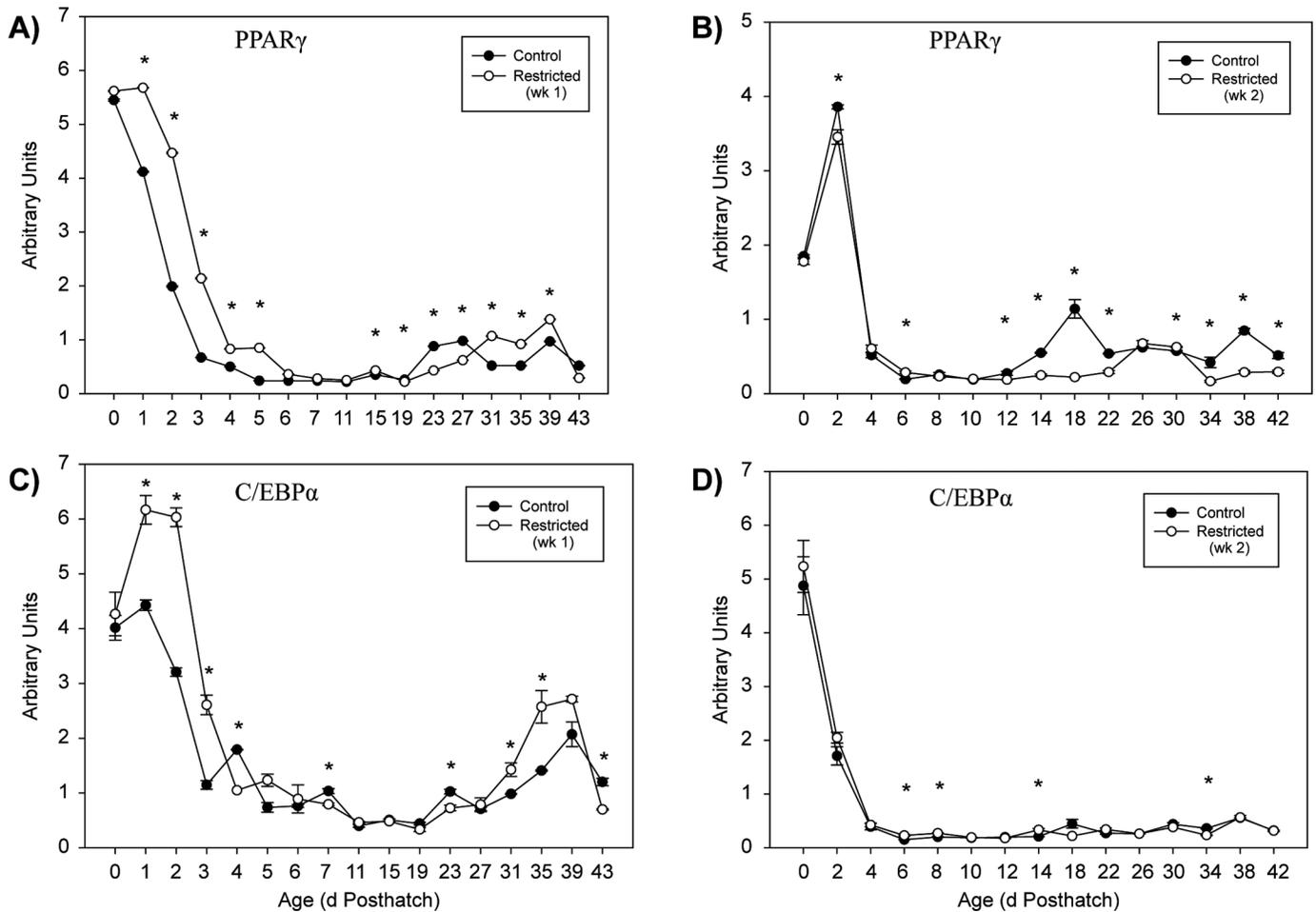


Figure 1. Expression of peroxisome proliferator-activated receptor gamma (PPAR γ) in the pectoralis major muscle of A) control full-fed and wk 1 feed-restricted birds, and B) control full-fed and wk 2 feed-restricted birds from hatch (d 0) through 43 or 42 d of age, respectively. Expression of CCAAT/enhancer-binding protein alpha (C/EBP α) in the pectoralis major muscle of C) control full-fed and wk 1 feed-restricted birds; and D) control full-fed and wk 2 feed-restricted birds from hatch (d 0) through 43 or 42 d of age, respectively. Each point represents the mean of 3 replicates of each sample and the bars indicate the SEM. *Indicates a significant difference ($P < 0.05$).

Cellular Imaging Center, The Ohio Agricultural Research and Development Center, Wooster). The real-time qPCR was performed with the following conditions: denaturation (94°C for 15 min), amplification and quantification (35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s), and final extension at 72°C for 5 min. The melting curve program was 52°C to 95°C, 0.2°C/read, and a 1-s hold. The relative level of gene expression was calculated using the standard curve for each target gene as described previously by Liu et al. (2006). Standard curves were constructed for each gene and GAPDH with serial dilutions of the purified PCR products of PPAR γ , C/EBP α , and GAPDH. The mole amount of sample cDNA for each gene from each treatment was interpolated from the corresponding standard curve. All of the sample concentrations fell within the values of the standard curves. The mole amount of each cDNA product was then normalized across sampling times using GAPDH expression as the normalizing gene with the sample concentration being divided by the GAPDH mole value. The resulting value is reported

as an arbitrary unit as described in Liu et al. (2006). Randomly selected samples from all real-time qPCR reactions were resolved by agarose gel electrophoresis to ensure gene amplification specificity. A negative control, a well with no template, was included in each PCR reaction to detect possible contamination.

Statistical Analysis

All statistical analyses were performed using the PROC GLM of SAS (2010, SAS Institute Inc., Cary, NC) to determine differences between the control and feed restriction treatments at each sampling time. Differences between the means were evaluated using a Student's t -test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The immediate posthatch period in broilers is a period of maximal muscle growth including the pectoralis

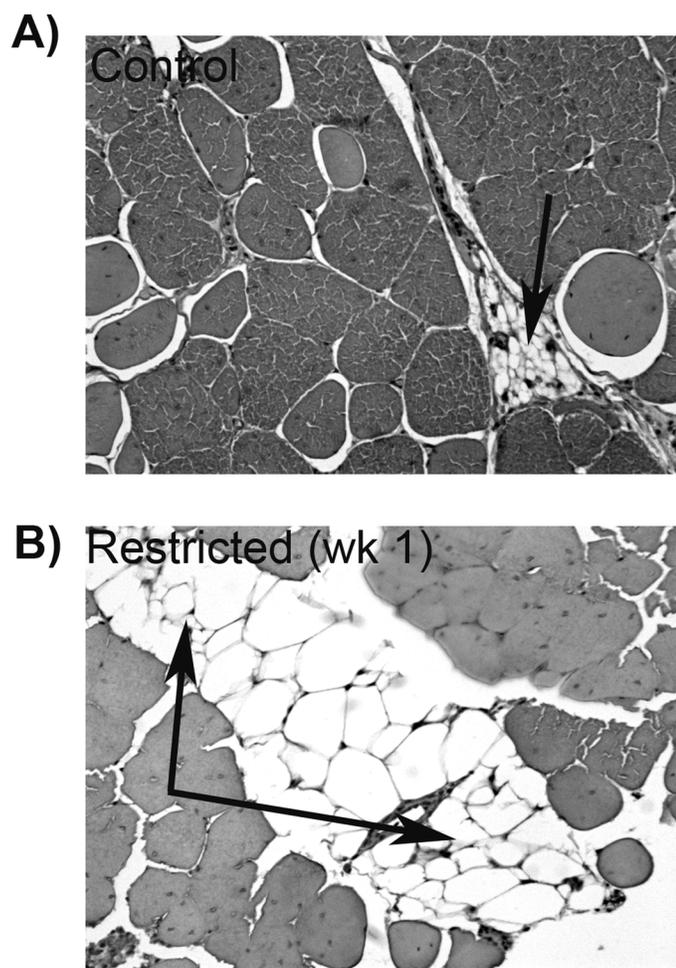


Figure 2. Morphology of pectoralis major muscle fat depots at 39 d of age in the A) control full-fed, and B) wk 1 feed-restricted chicks. The arrows highlight the fat depots in both the control and feed-restricted pectoralis major muscle. Scale bar = 50 μ m.

major muscle (Halevy et al., 2000). Myogenic satellite cells mediate the immediate posthatch period of muscle growth by donating their nuclei to existing muscle fibers leading to increased protein synthesis and muscle mass accretion through the hypertrophy of existing muscle fibers. In commercial hatcheries, chicks often must rely on nutrients from the yolk during the hatching and immediate posthatch and transport period with not being exposed to feed for 24 h or longer after hatch. During this period of time, the satellite cells have been shown to be sensitive to nutrition (Halevy et al., 2000; Mozdziaik et al., 2002). Using isolated broiler pectoralis major satellite cells, Powell et al. (2013) demonstrated that both the proliferation and differentiation of the satellite cells were sensitive to nutrition. Furthermore, nutritional status has been shown to induce the expression of adipogenic genes in vitro in broiler-derived pectoralis major muscle satellite cells (Powell et al., 2014). The transdifferentiation of satellite cells to an adipogenic cellular lineage will change fat deposition and the fat-to-lean composition in the muscle (Sordella et al., 2003; Quinn, 2008).

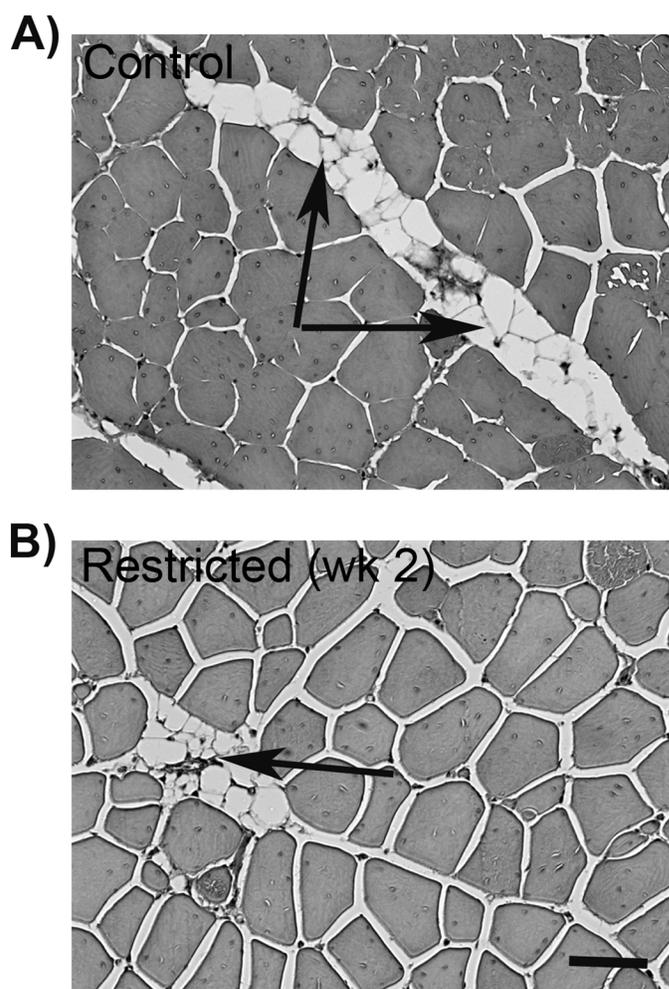


Figure 3. Morphology of pectoralis major muscle fat depots at 42 d of age in the A) control full-fed, and B) wk 2 feed-restricted chicks. The arrows highlight the fat depots in both the control and feed-restricted pectoralis major muscle. Scale bar = 50 μ m.

Expression of PPAR γ and C/EBP α

The expression of the adipogenic genes PPAR γ and C/EBP α was affected by the timing of the feed restriction (Figure 1). Both PPAR γ and C/EBP α have been shown to be involved in the transdifferentiation of muscle cells into adipocytes (Hu et al., 1995; Yu et al., 2006). In the wk 1 restricted birds, the feed restriction resulted in higher PPAR γ expression in the pectoralis major muscle compared with the control muscle during the first week posthatch (Figure 1A). Although expression of PPAR γ was low beginning at d 6 through the duration of the trial, PPAR γ expression was higher in the restricted birds from 31 through 39 d of age. When the feed restriction was moved to wk 2, the increase in PPAR γ expression in the feed restricted group was not present and the expression of PPAR γ in the control muscle was higher than the feed-restricted birds at 12 through 22 d of age and 34 to 42 d of age (Figure 1B). Expression of C/EBP α was elevated in the wk 1 feed restricted pectoralis major muscle during the first 3 d posthatch, and at 31 and 35 d of age (Figure 1C).

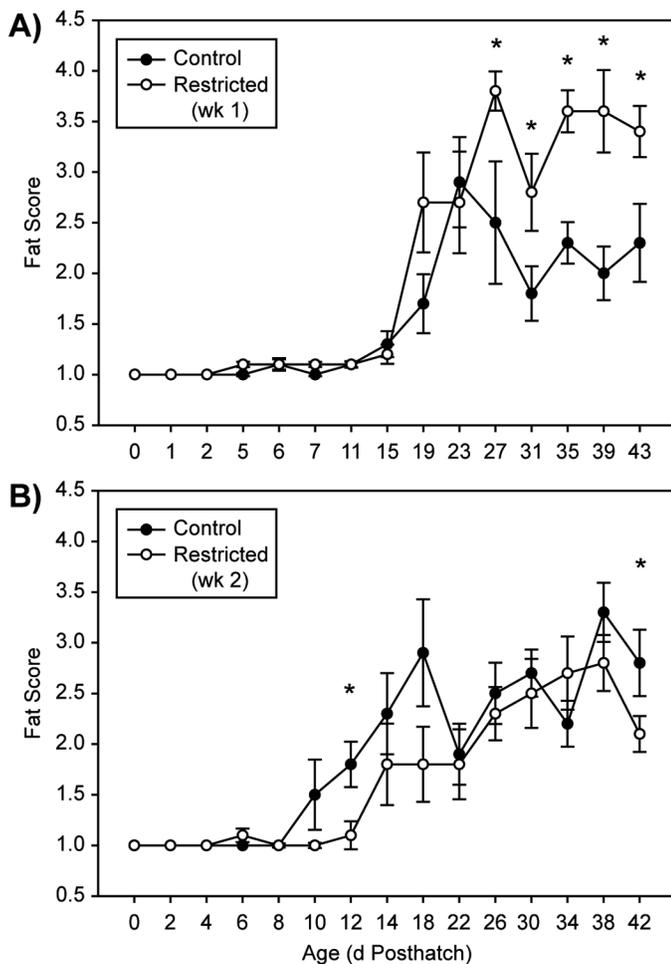


Figure 4. Morphometric analysis of fat depot morphology within the pectoralis major muscle of control full-fed and feed-restricted chicks from hatch (d 0) through 43 or 42 d of age. A) Control full-fed and wk 1 feed-restricted birds, and B) control full-fed and wk 2 feed-restricted birds. Fat depot morphology scores ranged from 1 (no observable fat depots) to 5 (extensive fat depots). Each data point represents the mean score from 4 trained evaluators who scored 8 different samples per treatment for each age, and the bars indicate the SEM. *Indicates a significant difference ($P < 0.05$).

In the wk 2 feed-restricted birds, the expression of *C/EBP α* in general was not altered in its expression from the control (Figure 1D).

Effect of the Timing of the Feed Restriction on Fat Deposition within the Pectoralis Major Muscle

At each sampling time, pectoralis major muscle morphological structure was microscopically evaluated for adipose deposition. In the wk 1 restricted pectoralis major muscle at 31 and 39 d of age, the fat depots were extensive compared with the control muscle. Figure 2 shows the distribution of the fat depots in the control and feed-restricted birds pectoralis major muscle at 39 d of age. In the wk 2 restricted birds, extensive fat depots were not observed and Figure 3 illustrates the deposition of fat in the control and restricted pectoralis major muscle at 42 d of age. The morphological images

were independently evaluated by a trained panel for the severity of the fat deposition (Figure 4). Fat deposition in the pectoralis major muscle from the wk 1 restricted birds by 27 d of age had more extensive fat depots compared with the pectoralis major muscle isolated from the control full-fed birds for the duration of the study (Figure 4A). In contrast, in the pectoralis major muscle isolated from the wk 2 feed-restricted birds, fat deposition was increased in the control full-fed muscle at 13 and 42 d compared with the feed restricted muscle (Figure 4B). At no time during the course of the study was the wk 2 feed restricted pectoralis major muscle evaluated as having more fat deposition than that observed in the control.

In summary, the results from the present study demonstrate that the timing of an immediate posthatch feed restriction does affect the expression of adipogenic genes and the deposition of fat within the pectoralis major muscle. Feed restrictions administered the first week posthatch result in increased expression of the adipogenic genes primarily during the first week after hatch and increased fat deposition. Moving the feed restriction to wk 2 removed all the effects on the expression of the adipogenic genes and the control full-fed birds were observed to have more extensive fat depots within the pectoralis major muscle. These data demonstrate that the appropriate nutritional regimen after hatch is important in regulating fat deposition occurring within the broiler pectoralis major muscle. The increase in fat deposition in the pectoralis major muscle with the wk 1 feed restriction coincides with the period of maximal satellite cell activity (Halevy et al., 2000). During this time the satellite cells are sensitive to nutritional regimen. As shown by Powell et al. (2014) broiler pectoralis major muscle satellite cells are able to trans-differentiate to an adipogenic lineage with altered nutrition. It is, therefore, important for broiler producers to understand the changes in cellular differentiation to alternative fates to develop appropriate management strategies to maintain the lean product quality associated with broiler breast meat.

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