Developmental regulation of adipose tissue growth through hyperplasia and hypertrophy in the embryonic Leghorn and broiler

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ABSTRACT The United States is a world leader in poultry production, which is the reason why achieving better performance and muscle growth each year is a necessity. Reducing accretion of adipose tissue is another important factor for poultry producers because this allows more nutrients to be directed toward muscle growth, but the effect of embryonic adipose growth on posthatch development has not been fully understood. The purpose of this study was to investigate the total DNA mass, morphological characteristics, differentiation markers, and triglyceride breakdown factors of embryonic adipose tissue, and their relation to hyperplastic and hypertrophic growth within layers (Leghorn) and meat-type chickens (broilers). After embryonic day (E) 12, broiler weight was significantly higher than Leghorn, and this trend continued throughout the rest of incubation and posthatch ($P < 0.05$). Neck and leg fat pad weights between the 2 breeds did not differ at most of the time points. A remarkable increase in total DNA mass was observed between E12 and E14 in both Leghorn and broilers ($P < 0.05$), indicating a high potential for hyperplastic growth during this time. Histological analysis revealed clusters of preadipocytes at E12; however, the majority of these cells differentiated by E14 and continued to grow until the time of hatch. The adipocyte sizes between both breeds did not generally differ, even though broilers are known to have larger adipocytes posthatch. Fatty acid-binding protein 4 expression levels in Leghorn and broilers continued to rise with each time point, which paralleled the expansion of mature adipocytes. Adipose triglyceride lipase was highly expressed at E20 and d 1 posthatch to mobilize triglyceride degradation for energy during hatching. Thus, embryonic chicken adipose tissue was found to develop by hyperplastic mechanisms followed by hypertrophy. At embryonic stages and early posthatch, layer- and meat-type chicken adipose growth does not differ, which suggests breed differences occur posthatch.

Key words: embryonic adipose, total DNA mass, adipocyte hyperplasia, adipocyte hypertrophy, differentiation

INTRODUCTION

The United States is one of the leading producers and exporters of poultry products in the world, which is the reason why poultry producers are constantly searching for ways to maximize egg production in layers and muscle accretion in broilers (USDA, 2012). Genetic selection for performance and breast yield has become the main focus of the poultry industry (Abdullah et al., 2010). However, minimizing adipose accumulation has surfaced as a new target in the poultry industry due to the fact that small gains in feed conversion result in high production gains (Sizemore and Siegel, 1993). Broilers are known to have a higher adipose weight compared with layers, such as Leghorns, because their accelerated growth rates are accompanied by more muscle growth, but also more fat accretion (Emmerson, 1997). Insufficient information has been collected regarding embryonic adipose growth in both Leghorn and broilers to determine whether adipose development patterns differ at this stage and have an effect on posthatch fat accretion.

Once the preadipocyte pathway is determined for a mesenchymal stem cell, maturation and growth is carried out by coordinated actions and signals. Hyperplasia, an increase in cell number, and hypertrophy, an increase in cell size, are the 2 mechanisms by which embryonic adipose tissue grows. In embryonic adipose development, hyperplastic growth of preadipocytes dominates, and hypertrophic growth is subsequent to fill the established cells with lipid (Jo et al., 2009). Adipokines and other signaling molecules, working at both the local and systemic level, promote or inhibit hyperplastic and hypertrophic mechanisms to regulate growth, along with nutrient availability from the yolk (Kershaw and Flier, 2004; Moran, 2007). Preadipocyte differentiation and lipid gene regulation are controlled

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by certain key transcription factors, such as peroxisome proliferator-activated receptor γ (PPAR-γ). One such gene regulated by PPAR-γ is fatty acid-binding protein 4 (FABP4), which mediates transport and metabolism of lipids within adipocytes (Damcott et al., 2004). As differentiation progresses, a dramatic increase in the expression of FABP4 is seen (Billon et al., 2007). This differentiation marker is linked to hypertrophy by stimulating the transport of fatty acids for triglyceride (TG) synthesis to fill the adipocyte-storage capacity (Sarruf et al., 2005).

Net accumulation of fat is the balance between TG synthesis and breakdown. Triglyceride breakdown is catalyzed by the rate-limiting enzyme, adipose triglyceride lipase (ATGL), also known as desnutrin, followed by actions of hormone-sensitive lipase and monoacylglycerol lipase (Villena et al., 2004; Yang et al., 2013). Adipose triglyceride lipase hydrolyzes the first bond of a stored TG to release a fatty acid and diacylglycerol (Zimmermann et al., 2004). Two conserved domains were identified within adult avian species. A patatin domain exhibits lipase activity, whereas a hydrophobic domain binds lipid droplets (Lee et al., 2009). Relatively little, if any, information has been collected regarding ATGL mechanisms within the embryonic chicken.

Overall, hyperplasia and hypertrophy in embryonic chicken adipose tissue along with the influence of differentiation markers and TG breakdown factors have not been extensively studied, which leaves the effect on posthatch performance and development to be unknown. Therefore, the aim of the current study was to understand adipocyte growth mechanisms of hyperplasia and hypertrophy throughout embryonic incubation and compare the dynamics of adipose growth between layer- and meat-type chickens. This will provide fundamental information on the morphology, proliferation, and differentiation of adipocytes and potential similarities or differences during embryonic development of both breeds.

**MATERIALS AND METHODS**

**Birds and Adipose Samples**

Fertile Leghorn (layer-type) eggs were obtained from The Ohio State University poultry house (Lane Avenue), and fertile broiler (meat-type) eggs were obtained from Ridgway Hatcheries (La Rue, OH). Eggs were incubated and turned through a 90° arc every 2 h. At d 12, 14, 16, 18, and 20 of incubation (E12, E14, E16, E18, and E20), eggs were removed from the incubator, and 15 embryos from each breed were extracted with forceps and weighed. At d 1 posthatch (D0), 15 chicks from each breed were humanely euthanized, in accordance with the Institutional Animal Care and Use Committee at The Ohio State University, and weighed. Fat pads on both sides of the neck and leg of each embryo or chick were excised and weighed (Figure 1).

The adipose fat pads of the first 5 embryos or chicks collected from each time point were fixed in 10% neutral buffered formalin and designated for histology. The tissues of the second 5 embryos or chicks collected from each time point were immediately stored at −80°C for Western blot analysis. The adipose tissues of the last 5 embryos or chicks were immediately stored at −80°C and used for determining total DNA concentration.

**Visualization of Embryonic Fat Pads**

Chicken embryos were removed from their eggs and washed with PBS several times. Then, embryos with feathers were further processed to remove the feathers by using forceps. Cleaned embryos were fixed in 10% neutral buffered formalin for 48 h. The fixed embryos were transferred into a 1% KOH solution for 16 to 48 h, depending on the size of embryo, to visualize the fat pads by clearing skin and muscle tissues. Then, the embryos were transferred and kept in 100% glycerol until a picture was taken (Figure 1).

**Total DNA Content**

The adipose sample from the neck or leg was lysed in a volume of cell lysis buffer (200 mM NaCl, 50 mM Tris, 10 mM EDTA, 1% SDS; pH 8.0) appropriate for the weight of the fat pad (300 μL/10 mg) in a 55°C water bath for 4 h with Proteinase K (1.5 μL/300 μL of cell lysis buffer) previously added. To remove residual protein, 300 μL of phenol-chloroform-isoamyl alcohol was added to the lysates, vortexed, and centrifuged at 12,000 × g for 2 min at 4°C. Then, the supernatant was extracted and 100 μL of 7.5 M ammonium acetate was added to the samples, vortexed, and centrifuged at 12,000 × g for 10 min at 4°C. The supernatant was removed; and 1 vol of isopropyl alcohol was added, inverted about 50 times, and centrifuged at 12,000 × g for 5 min at 4°C. The resulting pellet was washed with 70% ethanol and dried. The DNA was resuspended in 100 μL of Tris-EDTA (TE) buffer (10 mM Tris, 1 mM EDTA; pH 8.0) containing the RNase A (10 μg/mL) and stored at 4°C overnight. The next day, 200 μL of TE buffer was added with 300 μL of phenol-chloroform-isoamyl alcohol, vortexed, and centrifuged at 12,000 × g for 2 min at 4°C. Then, the supernatant was extracted and 70 μL of 7.5 M ammonium acetate was added to the samples, vortexed, and centrifuged at 12,000 × g for 10 min at 4°C. The supernatant was removed; and 1 vol of isopropyl alcohol was added, inverted about 50 times, and centrifuged at 12,000 × g for 5 min at 4°C. The resulting pellet was washed with 70% ethanol and dried. The DNA was resuspended in 50 μL of TE buffer, and the DNA concentration was measured with a Nanodrop spectrophotometer (Thermo Scientific, Waltham, MA). Total DNA concentrations of the neck and leg fat pads were calculated with this data.
Adipocyte Histological Processing

Fat pads on each side of the neck and leg were fixed in 10% neutral buffered formalin, and then replaced with 70% ethanol 48 h later. Tissues were dehydrated with 95% ethanol (2 × 40 min) and 100% ethanol (3 × 40 min), cleared in xylene (2 × 60 min), and embedded in paraffin. Microscope slides were prepared with 3 or 4 serial 8-μm thick slices using a microtome. Four slides were prepared for either the neck or leg of each animal. Slides were deparaffinized in xylene (3 × 4 min) and hydrated with ethanol and distilled water (dH₂O). Ethanol hydration consisted of 100% (2 × 3 min), 95% (1 × 3 min), and 70% (1 × 3 min). Final hydration was performed in dH₂O (1 × 3 min). Slides were placed in Gill’s hematoxylin (Vector Laboratories, Burlingame, CA) for 7 min and washed with running tap water for 7 min. Then, slides were dipped into acid ethanol (1% HCl in 95% ethanol) 3 times to remove residual hematoxylin. Slides were washed with dH₂O (2 × 2 min), placed in eosin for 2 min, dehydrated with 95% ethanol (2 × 2 min) and 100% ethanol (2 × 3 min), cleared with xylene (2 × 3 min), and a permanent coverslip was mounted with Permount Mounting Medium (Fisher Scientific, Waltham, MA). Stained slides were observed and imaged with AxioCam MRc 5 (Zeiss, Thornwood, NY), and ImageJ software (NIH ImageJ 1.47; http://imagej.nih.gov/ij) was used to determine cell size. Average adipose tissue cross-sectional area (CSA) was calculated by taking the area of a large portion of cells and dividing this by the total number of cells found within the area. At least 800 cells were evaluated per animal.

Western Blot Analysis

Protein lysates were isolated from neck fat using a lysis buffer (62.5 mM Tris, pH 6.8, and 1% SDS). Lysates were vortexed to ensure all cells were lysed, and the mixture was centrifuged at 13,000 × g for 1 min at 24°C. The supernatant containing the proteins was extracted and transferred to a clean tube. Then, 100 μL of 2× Laemmli buffer (62.5 mM Tris, pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 12.5% glycerol, and 0.05% bromophenol blue; Bio-Rad Laboratories, Hercules, CA) was added to 100 μL of the protein mixture. Gel percentage was chosen by target protein size, with smaller proteins being on higher percentage gels and larger proteins on lower percentage gels. Coomassie blue staining was used to visualize all proteins within the adipose samples. Proteins were wet-transferred to Immobilon Transfer membranes (Millipore, Billerica, MA). Membranes were blocked in 5% nonfat dry milk in Tris-buffered saline-Tween (TBST; 20 mM Tris, 150 mM NaCl, pH 7.4, plus 0.1% Tween 20) for 30 min at room temperature. Next, the membranes were incubated for 1 h with a goat antibody against FABP4 (R&D systems, Minneapolis, MN) and β-actin (1:2,500 dilution;
Santa Cruz Biotechnology Inc., Santa Cruz, CA), or with a rabbit antibody against human ATGL (1:1,000 dilution; Cell Signaling Technology, Danvers, MA). The membranes were washed 10 times in TBST, incubated in horseradish peroxidase-conjugated antibodies (1:2,500 dilution; Santa Cruz Biotechnology Inc.) for 1 h at room temperature, and washed another 10 times in TBST. Proteins were detected using ECL plus (Amersham Biosciences, Piscataway, NJ) and exposed to Biomax x-ray film (Amersham Biosciences).

**Statistical Analysis**

Descriptive statistics were calculated using the MEANS procedure of SAS (SAS Institute, 2009). A general linear model was used to detect significant differences among chicken breeds and age groups. Significant differences of mean values among chicken breeds and age groups were detected by the probability difference, and mean values were separated at the level of 5%. Results are presented as least squares means together with standard errors of the least squares means.

**RESULTS**

**Embryo, Body, and Adipose Weights**

Embryo weight (EW) and BW between Leghorn and broilers at each time point are presented in Figure 2. Flock ages and egg characteristics were not measured for the current study, which may result in some variability in data depending on factors, such as hen age and yolk size. From E12 to E20, embryos of both breeds continually gained a significant amount of weight ($P < 0.05$), but E18 to E20 in Leghorn (19.9 vs. 39.3 g) and broilers (31.2 vs. 50.3 g) demonstrated the greatest increase in weight. Leghorn BW decreased slightly from E20 to D0, but was not significant (39.3 vs. 37.8 g). Broiler BW had a significant decrease from E20 to D0 (50.2 vs. 46.9 g; $P < 0.05$). When comparing Leghorn and broilers, no significant difference in EW at E12 (5.34 vs. 6.79 g; $P > 0.05$) was observed. Broiler EW and BW were significantly higher than Leghorn at E14 (11.0 vs. 14.9 g; $P < 0.05$) and at each subsequent time point ($P < 0.05$). A significant difference in EW occurred at E18 between Leghorn and broiler (19.9 vs. 31.2 g; $P < 0.05$).

Neck fat pad weights, leg fat pad weights, and neck and leg fat pad weight percentage relative to EW or BW are displayed in Figure 3. Neck fat pad weight was noted for Leghorn and broilers (0.243 vs. 0.196 and 0.209 vs. 0.169 g, respectively; $P < 0.05$), whereas leg fat pad weights varied at E18 and D0 when broiler was higher (0.141 vs. 0.170 and 0.181 vs. 0.231 g, respectively; $P < 0.05$). Leghorn and broiler fat weight percentage relative to EW or BW was not different at E12. Neck fat pad weight percentage relative to EW or BW after E12 was significantly lower in broiler due to their larger EW and BW. Leg fat pad weight percentage relative to EW or BW after E12 was lower in broiler until D0, which equaled Leghorn (0.48 vs. 0.49%).

**Total DNA Mass**

Total DNA mass within the neck fat pads and leg fat pads of Leghorn and broiler are shown in Figure 4. Neck DNA mass was approximately 4.0-fold higher in Leghorn from E12 to E14 and 3.6-fold higher in broilers from E12 to E14 (7.69 vs. 31.2 and 8.27 vs. 30.1 μg, respectively; $P < 0.05$). This trend was also observed in leg DNA mass from E12 to E14 with a 4.4-fold increase in Leghorn and a 2.6-fold increase in broilers (8.24 vs. 36.6 and 12.9 vs. 33.9 μg, respectively; $P < 0.05$). Leghorn DNA mass in both neck and leg fat pads increased until E18, which marked the time point when the levels achieved a steady state. The same trend was noticed in broiler DNA mass, but a slight increase was observed at D0 in neck and leg. Between Leghorn and broilers, DNA mass did not differ at any time point in the neck or leg.

**Adipocyte Size and Characteristics**

Histological images of E12 neck and leg fat pads in Leghorn and broilers are presented in Figure 5A. Clusters of nuclei demonstrated the groups of preadi-
pocytes that would eventually differentiate into multi-
locular adipocytes under optimal circumstances. Sev-
eral multilocular or small unilocular adipocytes were
visible; therefore, the adipocyte size at E12 could not
be measured. Figure 5B displays histological images of
adipocytes in the neck and leg of both breeds at all of
the older time points. A clear expansion of adipocyte
size is evident from E14 to E20 in each breed, and
a slight decline in size is noticeable from E20 to D0,
which resembles the reduction in EW or BW and fat
pad weight at this point. Adipose tissue CSA of neck
and leg fat pads in both breeds is shown in Figure 5C.
Similar to the histological images, a continuous increase
in adipose tissue CSA was noted from E12 to E20 along
with a decline at D0 in the neck and leg fat pads of each
breed. Adipose tissue CSA of Leghorn leg was larger
than broiler at E14 (566 vs. 463 μm; \( P < 0.05 \)), E16
(794 vs. 676 μm; \( P < 0.05 \)), and E18 (1,058 vs. 973 μm;
\( P < 0.05 \)), but Leghorn was smaller than broiler at D0
(1,086 vs. 1,137 μm; \( P < 0.05 \)).

**Adipogenic and TG Breakdown Markers**

Protein expression by Western blot analysis of FABP4
in the mouse, chicken, cattle, and pig and adipose-spec-
ificity of FABP4 are shown in Figure 6A. Although the
FABP4 signal was not strong for the chicken compared
with other species, it was still detectable within the
adipose tissue, validating the usefulness of this FABP4
antibody for studying chicken FABP4 proteins. Similar
to mouse, bovine, and porcine FABP4 proteins (Shin
et al., 2009), the adipose-specific nature of FABP4 was
displayed in the abdominal fat and subcutaneous fat of
the broiler, whereas no expression was detected with-
in the kidney, liver, lung, heart, pectoralis muscle, or
tibialis muscle. Figure 6B displays FABP4 and ATGL

![Figure 3](image-url)

**Figure 3.** Comparison of fat pad weights in the neck (A) and in the leg (B) with neck fat percentage (C) and leg fat percentage (D) relative
to embryo weight or BW between Leghorn and broilers at different embryonic days (E; \( n = 15 \) per time point per group). Bars represent mean ±
SEM. Significance (\( P < 0.05 \)) indicated by different letters (a–g).
expression levels in Leghorn and broiler from E12 to D0. The FABP4 was expressed at increasing levels with each time point in Leghorn and broilers, correlating to growing numbers of mature adipocytes in the animal. At E20 and D0, dramatic increases in expression of ATGL were exhibited in both breeds. This is the point when lipids must be mobilized to provide energy for the hatching process; therefore, ATGL becomes functional to mobilize lipid within the adipose tissue, and a decline in BW and fat pad weight takes place (Lee et al., 2009). Free fatty acids released by the adipocyte are shuttled to the liver. The free fatty acids that are not re-esterified are used as a primary energy source for fasting periods, such as directly after hatch (Sun et al., 2011). In the poultry industry, feed is often withheld following hatching because of sexing, vaccination administration, and transportation. This period causes weight loss in the chicks as they use lipids from the yolk or adipose tissue. Thus, upregulation of ATGL occurs in response to this feed restriction to promote lipolysis and release fatty acids for energy (Lee et al., 2009). When assessing differences between breeds, broiler EW was significantly higher at E14 and each subsequent time point \((P < 0.05)\). The gap in EW widened as both breeds reached E18, with broilers exceeding Leghorn, possibly because of larger muscle accretion from genetic selection, and this trend did not shrink after hatch. Interestingly, neck and leg fat pad weights did not greatly differ between Leghorns and broilers, and broilers had lighter neck fat pad weights at E20 and D0. When the fat pad weights were compared with total EW or BW, Leghorns usually had a higher fat percentage within the body. Due to extensive genetic selection within broiler strains, accelerated growth of the pectoralis muscle is noted even during embryonic stages, which can offset the adipose to BW ratio (Emmerson, 1997).

**DISCUSSION**

Meat-type chicken embryonic growth rates begin to accelerate past layer-types around the middle of embryonic development. This early rapid growth may be attributed to a larger yolk mass within the meat-type egg, which corresponds to higher nutrient availability (Ho et al., 2011). Yet, the pathways and targets of these nutrients have not been clarified. Fat pads in both breeds can be seen with the naked eye starting at E9. In the current study, EW gain in Leghorn and broilers was constant until a considerable leap in EW occurred in both breeds from E18 to E20, which is attributed to incorporation of the yolk into the embryo. Between E20 and D0 in both breeds, BW and fat pad weights declined, which was due to increased energy expenditure during hatching. Chickens have a high demand for energy during the hatching process; therefore, ATGL becomes functional to mobilize lipid within the adipose tissue, and a decline in BW and fat pad weight takes place (Lee et al., 2009). Free fatty acids released by the adipocyte are shuttled to the liver. The free fatty acids that are not re-esterified are used as a primary energy source for fasting periods, such as directly after hatch (Sun et al., 2011). In the poultry industry, feed is often withheld following hatching because of sexing, vaccination administration, and transportation. This period causes weight loss in the chicks as they use lipids from the yolk or adipose tissue. Thus, upregulation of ATGL occurs in response to this feed restriction to promote lipolysis and release fatty acids for energy (Lee et al., 2009). When assessing differences between breeds, broiler EW was significantly higher at E14 and each subsequent time point \((P < 0.05)\). The gap in EW widened as both breeds reached E18, with broilers exceeding Leghorn, possibly because of larger muscle accretion from genetic selection, and this trend did not shrink after hatch. Interestingly, neck and leg fat pad weights did not greatly differ between Leghorns and broilers, and broilers had lighter neck fat pad weights at E20 and D0. When the fat pad weights were compared with total EW or BW, Leghorns usually had a higher fat percentage within the body. Due to extensive genetic selection within broiler strains, accelerated growth of the pectoralis muscle is noted even during embryonic stages, which can offset the adipose to BW ratio (Emmerson, 1997).

Hyperplasia and preadipocyte differentiation are some of the first mechanisms observed during adipose tissue development (Jo et al., 2009). This increase in preadipocyte number ultimately lays the foundation for differentiation to mature adipocytes and accumulation of lipid. As the number of cells continues to rise, total DNA mass is directly proportional to the number of cells within the tissue because each cell contains the same amount of DNA, and therefore indicates points of hyperplastic growth (Guo et al., 2011). In Leghorn and broilers, a significant increase \((P < 0.05)\) in total DNA mass was observed from E12 to E14 in both neck and leg fat pads. An increase in total DNA mass between E12 and E14 could illustrate induced hyperplasia followed by a slowing of cell number expansion as embryos reach E18. Leghorn DNA mass plateaued after E18, but broiler DNA mass had a minor increase at D0, which implied another small expansion of cell number. Substantial gatherings of preadipocytes were found in E12 Leghorn and broiler adipose tissue, indicating low levels of differentiation. Mature adipocytes with smaller
Figure 5. Histological images of embryonic day (E) 12 neck and leg adipose tissue at 200× (A) and subsequent time points [E14 to d 1 post-hatch (D0)] for Leghorn and broilers at 100× (B). Scale bar = 50 μm. Further comparison of cross-sectional area (CSA) of the neck fat pad and leg fat pad (C) in the Leghorn and broilers (n = 5 per group). Bars represent mean ± SEM. Significance (P < 0.05) indicated by different letters (a–h).
pockets of preadipocytes were noticeable as the embryos grew to E14. During this stage of incubation, the adipose tissue in both breeds is undergoing an expansion of cell number, and preadipocytes are rapidly differentiating into mature, lipid-containing adipocytes (Speake et al., 1996).

As differentiation of preadipocytes progresses, cells first become multilocular with several small vesicles of lipid, and then they expand and join to form 1 lipid droplet, termed unilocular. The transition from multilocular to unilocular adipocytes occurs at varying times across species. Hausman and Kauffman (1986) discovered that fetal to d 1 postnatal pigs possessed multilocular adipocytes and did not develop any unilocular adipocytes until d 3. At d 9, the majority of adipocytes were unilocular, whereas multilocular cells were found sparingly. When examining adipose tissue of 1-d-old calves, unilocular adipocytes are present with relatively no multilocular adipocytes (Smith et al., 2004), demonstrating earlier maturation of adipocytes. Chickens, as well as other avian species, require an adequate amount of lipid transferred from the yolk to hatch at the end of incubation (Hausman and Kauffman, 1986). After E12, TG begin to rapidly deposit within subcutaneous adipose tissue in chicken embryos (Speake et al., 1996). The FABP4 binds and carries long-chain fatty acids to maintain accumulation of TG within maturing adipocytes (Shi et al., 2010). A continuous gain in adipocyte size was noted from E14 to E20 in Leghorn and broilers. Then, adipocyte size slightly receded at D0 due to previously explained mechanisms of ATGL. Jo et al. (2009) revealed a strong positive correlation between fat pad mass and volume-weighted mean cell size in mice, indicating that hypertrophy is the main contributor to increases in adipose tissue mass. Although fat pad weight and DNA were the same between Leghorn and broilers at E16, CSA was significantly higher in Leghorn than broiler, especially in the neck. This may be attributed to faster maturation

Figure 6. Western blot analysis for (A) fatty acid-binding protein 4 (FABP4) expression demonstrated in the mouse, chicken, cattle, and pig with normalization to β-actin and adipose specificity shown in the broiler compared with other tissues. The 2 fat samples depicted are abdominal fat (AF) and subcutaneous fat (SQ). No expression of FABP4 was found in the kidney (K), liver (Li), lung (Lu), heart (H), pectoralis muscle (PM), or tibialis muscle (TM). (B) Leghorn and broiler expression levels of FABP4 and adipose triglyceride lipase (ATGL) from embryonic day (E) 12 to d 1 posthatch (D0). Coomassie blue protein staining was used as a standard instead of using cytoskeletal proteins, including β-actin or α-tubulin, which showed variation in the amount of these proteins during embryonic adipose development that is usually accompanied with dynamic changes in cytoskeleton structures.
of adipocytes in the Leghorn accompanied by many small, immeasurable preadipocytes that still contribute to DNA mass and fat pad weight if present in high enough quantities. Contrarily, broiler adipocytes may possess a slow, steady growth rate with more differentiated preadipocytes, but the same number of adipocytes is present in the fat pad.

In summary, total DNA mass accompanied by increases in adipocyte size exemplify the 2 mechanisms of hyperplasia and hypertrophy that contribute to adipose mass. To our knowledge, this is the first study investigating the fundamental aspects of adipose growth and development in chicken embryos. In conclusion, it was determined that, although meat-type chickens possess larger EW, weight and development of embryonic adipose tissue is not noticeably different between meat- and layer-type chickens. Therefore, posthatch events are most likely the reason for differences in adipose weights between the 2 breeds. More information on molecular pathways of embryonic chicken adipose tissue growth must be collected along with investigation of posthatch development differences of layer- and meat-type chickens to apply this knowledge for reducing fat accretion in the poultry industry.

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