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Similar to the result of the liver, ACRB on E14 had significantly higher DHA ($P = 0.004$) and total n-3 FA ($P = 0.003$) compared to the other groups. Collins KE, Marks HL, Aggrey SE, Lacy MP, Wilson JL. Liver development and lipid deposition. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. This may be because COBB has higher $\Delta 6$ -desaturase activity. Kim JW, Fletcher DL, Campion DR, Gaskins HR, Dean RG. ME catalyzes the reaction that generates NADPH, which is used by FASN in synthesizing palmitate (35). Some evidence showed that ApoB also highly expressed in the yolk sac during embryonic development (32). Hu Q, Agarwal U, Bequette BJ, Kim JB, Spiegelman BM. Relative fat weight was higher on E18 than E14 ($P = 0.03$), and COBB embryos had higher fat tissue weight than ACRB on E18 ($P = 0.0002$). These results indicated that selection of growth does affect embryonic development of different breeds of chickens. In brief, saponification was carried out for all samples by adding 0.5 M methanolic KOH at 110 °C for 1 h, followed by methylation. Messenger RNA levels and transcription rates of hepatic lipogenesis genes in genetically lean and fat chickens. doi: 10.1167/iov.13-13457 PubMed Abstract | CrossRef Full Text | Google Scholar 28. Future studies should examine the expression of genes in protein synthesis and satellite cell specific myogenesis factors. Lipogenesis and fatty acid (FA) oxidation are important metabolic pathways in controlling hepatic triacylglycerol content and body fat accumulation (8, 9). doi: 10.1109/TPWRD.2010.2063718 CrossRef Full Text | Google Scholar 28. *Indicates sinusoidal capillaries. (2010) 26:258-67. (2007) 26:1122-36. All authors read and approved the final version of the manuscript. FA composition differences in the liver were largely affected by embryonic age (Table 6). The embryonic day and breed information are indicated in the upper right corner in each picture. doi: 10.1172/JCI15593 PubMed Abstract | CrossRef Full Text | Google Scholar 37. The ACRB chicks hatched with a lighter residual yolk sac as a percentage of chick weight. However, few studies have been conducted to understand the roles of breed and embryonic stage on fatty acid profiles and myogenic and lipogenic genes in key organs between selected and unselected chicken breeds during embryonic development. J Biol Chem. Fatty acid composition of the yolk from ACRB and Cobb broiler embryos at E14 and E18. The hedgehog signaling pathway is acknowledged for its function in inhibiting myogenesis (22). (2014) 55:424-39. Myogenesis in the chicken: the onset of differentiation of adult myoblasts is influenced by tissue factors. doi: 10.1079/WPS19620019 CrossRef Full Text | Google Scholar 3. Two cell lineages, myf5 and myf5-independent, participate in mouse skeletal myogenesis. Liver dominant expression of fatty acid synthase (FAS) gene in two chicken breeds during intramuscular-fat development. Expression of myogenic regulatory factors in chicken embryos during somite and limb development. The main effect of breed was shown in ApoB and MTP; the gene expression was significantly higher in ACRB. At E18, both n-6 and n-3 essential FAs, linoleic, and α -linolenic acids were also significantly ($P < 0.0001$) increased in both breeds, while saturated, arachidonic acid and DHA levels decreased. (2015) 10:e0122643. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. The interaction of ApoA-I and ABCA1 triggers signal transduction pathways to mediate efflux of cellular lipids. doi: 10.3382/ps/pew249 PubMed Abstract | CrossRef Full Text | Google Scholar 14. In their study, egg composition, conductance values, incubation duration, hatch performance, and yolk utilization were measured (6). The forward and reverse primers for the 12 genes are shown in Table 1. Egg characteristics and hatch performance of athens canadian random bred 1955 meat-type chickens and Cobb 500 broilers. (2014) 101:67-74. Res Vet Sci, Horton JD, Goldstein JL, Brown MS. (2015) 227:352-60. Embryo weight was recorded, and breast muscle, liver, and abdominal and thigh adipose tissues were removed and weighed. However, no information on embryo weight or organ/tissue weight was indicated in their comparison of ACRB and COBB broilers. However, lipid deposition on E18 was considerably higher than E14 regardless of the breeds. MYF6 promotes the expression of muscle proteins and other related muscle-specific genes through trans-activation (42). Sample Collection Yolk samples of both breeds on E0 were collected for lipid profiling. Egg, embryo, and relative embryo and tissue weight on embryonic days 14 and 18. doi: 10.1016/j.biochi.2013.12.020 PubMed Abstract | CrossRef Full Text | Google Scholar 33. ABCA-1, ATP-binding cassette 1; ACC, acetyl coenzyme A carboxylase; ACly, adenosine triphosphate citrate lyase; ApoB, apolipoprotein B; FASN, fatty acid synthase; MTP, microsomal triglyceride transfer protein; PPAR α , peroxisome proliferator-activated receptor α ; PPAR γ , peroxisome proliferator-activated receptor γ ; SREBP-1, sterol regulatory element-binding protein 1; Jensen-Ustad AP, Semenkovich CF. A comparison of key myogenic genes between ACRB and COBB is shown in Figure 2. Other molecules in the Wnt pathway include PAX3 and PAX7, which have similar yet distinct functions in regulating myoblast differentiation and expression of MYF5 and MYOD (21). In terms of age differences, the most pronounced difference was identified in oleic acid, which increased to a level 2.4–2.5 times higher in E18 in both breeds ($P < 0.0001$). In the present study, SREBP-1 showed higher expression in COBB embryos on E14 and E18, which may correspond to the elevated FASN on E18. Invest Ophthalmol Vis Sci. Six replicated samples were run in duplicate, and relative gene expression data were analyzed using the 2- $\Delta\Delta$ CT method (23). There are about 30% lipids in the egg yolk, which are mainly triacylglycerides, phospholipids, and cholesterol (10). The main effect of embryonic day was observed in ABCA-1, ApoB, MTP, PPAR- α , and SREBP; the expression of these genes was significantly higher on E18 when compared to E14. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. Zhao S, Ma H, Huang C, Zou S. Materials and Methods Eggs and Incubation The ACRB breeders were artificially inseminated with pooled semen 5 days before the incubation. Arrows indicate lipid deposit in hepatocytes. The y-axis is the percentage of the indicated fatty acid in total fatty acid of the designated tissue. Because ACRB was maintained with random breeding, it has not been heavily selected for body weight gain as commercial broilers, and the genetics of ACRB has been maintained to eliminate commercial selection. Daval S, Lagarrigue S, Douaire M. Hepatic gene expression during embryonic development has been less studied. In the initial egg yolk, COBB chicken has lower monounsaturated FA levels but more n-6 and n-3 FAs. During development, ACRB has higher n-6 FA content in the yolk compared to COBB. Figure 2. PAX3 and PAX7 promote the proliferation of either satellite cells or C2C12 myoblasts (21); MYF5 and MYOD1 are associated with myoblast proliferation, and MYOG regulates myoblast differentiation (41). doi: 10.1371/journal.pone.0004475 PubMed Abstract | CrossRef Full Text | Google Scholar 22. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins (2014). doi: 10.3382/ps.2014.03695 PubMed Abstract | CrossRef Full Text | Google Scholar 7. doi: 10.1016/j.devcel.2008.01.002 PubMed Abstract | CrossRef Full Text | Google Scholar 20. In mammals, there are three SREBP isoforms, designated as SREBP-1a, SREBP-1b, and SREBP-2. Egg weight on the sampling day was recorded, and the embryo was removed for further analyses. These results suggested that genetic selection has not only modified feed consumption and body composition after hatch but also affected lipogenesis and lipid metabolism within the egg. In posthatch development, other studies showed that COBB had larger breast and leg muscles and had a significantly greater fat pad, when compared to ACRB, but had smaller heart and liver as a percentage of body weights (4). Chickens only have one SREBP1 isoform, and it is highly homologous to the SREBP1a in mammals (13). Hepatic lipogenesis in broiler chickens with different fat deposition during embryonic development. Hepatic triglyceride homeostasis is maintained by regulating FA uptake, de novo lipogenesis, and FA export and/or oxidation (31). Christensen VL, Havenstein GB, Davis GS. (1985) 20:278–82. doi: 10.1051/gse:2000134 PubMed Abstract | CrossRef Full Text | Google Scholar 15. Muscle growth during embryonic development is called hyperplasia, which refers to increase of cell number. In embryonic stages, myoblasts proliferate and differentiate into myotubes, which form the myofibers. Ethics Statement All experiments were performed in accordance with the guidelines for the use of animal in research as stated by the Institutional Animal Care and Use Committee at the University of Georgia. The graph shows the means \pm SE. Table 1. Control of muscle cell-type specification in the zebrafish embryo by Hedgehog signaling. The hedgehog regulated oncogenes Gli1 and Gli2 block myoblast differentiation by inhibiting MyoD-mediated transcriptional activation. Lipids from yolk are absorbed by endocytosis of lipoprotein, as no receptors for free FAs have been found so far (27). Integrated functions of Pax3 and Pax7 in the regulation of proliferation, cell size and myogenic differentiation. Collins KE, Kiepper BH, Ritz CW, McLendon BL, Wilson JL. World's Poultry Sci J. RNA quantity and purity were determined using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA, USA). α -linolenic (18:3n-3), Wilkins (2014). doi: 10.3382/ps.2014.03695 PubMed Abstract | CrossRef Full Text | Google Scholar 7. doi: 10.1016/j.devcel.2008.01.002 PubMed Abstract | CrossRef Full Text | Google Scholar 20. In mammals, there are three SREBP isoforms, designated as SREBP-1a, SREBP-1b, and SREBP-2. Egg weight on the sampling day was recorded, and the embryo was removed for further analyses. 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