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keywords

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Introduction

The selection of broiler lines for increased growth and muscle development has been accompanied by significant anatomical, physiological and metabolic changes. As a consequence, physiological limitations have appeared, affecting both the robustness of the animals and the quality of the products (meat). In particular, in modern broiler lines, a decrease in muscle energy reserves, assessed by glycogen content in vivo, has been observed (Berri et al., 2001, 2007). Divergent selection on *Pectoralis major* muscle pHu allowed the creation of the pHu+ and pHu- lines, which differentiate on their glycogen reserves at the muscle level and represent a unique model to study the genetic and biological control of this trait in chicken. The present study aimed to describe the early mechanisms involved in the establishment of the pHu+ and pHu- phenotypes by monitoring between day 16 in ovo and 5 days post hatch the expression and/or activity of key enzymes involved in glycogen synthesis and degradation (glycogen synthase and glycogen phosphorylase) as well as several molecular actors related to carbohydrate metabolism.

Material and Methods

The study was performed on embryo and chicks from the two lines (pHu+/pHu-). *Pectoralis major* muscle sampling was performed on 16-day-old embryos (E16; N=7 pools of 3 individuals per line), hatched (D0) and 5-day-old (D5) chicks (at least N=12 par age and per line).

The *in vivo* glycogen content was estimated at each stage through the measurement of the glycolytic potential that reflects the glycogen that is in the tissue prior to slaughter as it considers the main intermediates of glycogen degradation (glucose-6-phosphate, free glucose, and lactate; Monin and Sellier, 1985). The glycogen phosphorylase activity was measured at D5 using the glycogen phosphorylase activity kit from abcam (ab273271). For cell signaling analyses, the muscle lysates were subjected to SDS-PAGE and Western blotting with the appropriate antibody. Bands were visualized with Infrared Fluorescence using the Odyssey Imaging System and quantified with the Odyssey imaging system software.

Data are presented as mean ± SEM. In case of normal residual and variance distribution they were subjected to ANOVA to assess line and stage effect and their interaction. In this case, the comparisons of means for each significant effect were performed using Fisher's least significant difference test. When the residuals were not normally distributed and variances were not homogeneous between groups, data were analyzed with the non-parametric Kruskal-Wallis test and a multiple comparison test. Differences were considered to be significant when p values were below 0.05.

Results and Discussion

The *in vivo* glycogen content was higher in pHu− than in pHu+ *Pectoralis major* muscles at hatching and 5 days post-hatch while no difference was observed in E16 embryos. Regardless of the line, the muscle lactate content increased from E16 to D5. Concerning muscle glycogen content, its evolution with time depends on the line: it increased between E16 and D5 in pHu- while it remained unchanged between E16 and hatching before increasing

phosphorylase (PYG), involved in glycogen synthesis and degradation, respectively, were studied. Five days after hatching, the level of GS phosphorylation was similar between the two lines. In contrast, PYG expression was higher in the muscle of pHu+ chicks compared to pHu-. In addition, specific PYG activity was more than 3-fold higher than in pHu- muscle at the same age (D5). Analyses are underway to define when during development (E16 or hatching) differences in PYG expression and activity are established between the two lines.

Conclusion and Implications

Differences in energy status between the pHu+ and pHu- lines appear very early in development, probably between E16 and hatching. Our results show for the first time that the differences in muscle glycogen content observed at hatching between the two lines are related to differences in glycogen degradation, which is much more pronounced in the pHu+ than in the pHu- line. The higher glycogen utilization observed in pHu+ chicks could be related to their greater propensity for protein synthesis and growth, which is expressed from the early stages of postnatal development (Métayer-Coustard et al., 2021).

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