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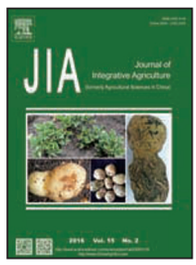

RESEARCH ARTICLE

Myofiber development during embryonic to neonatal development in duck breeds differing in muscle growth rates

LI Hui-fang[1*], SHU Jing-ting[1*], SHAN Yan-ju[1], CHEN Wen-feng[2], SONG Chi[1], XU Wen-juan[1]

[1] *Key Laboratory for Poultry Genetics and Breeding of Jiangsu Province, Institute of Poultry Science, Chinese Academy of Agricultural Science, Yangzhou 225125, P.R.China*

[2] *Jiangsu Tengdayuan Co. Ltd., Jiangyan 225538, P.R.China*

Abstract

Little is known about the muscle developmental patterns during embryonic to neonatal development in ducks. We investigated the developmental patterns in the lateral gastrocnemius muscles of Gaoyou and Jinding ducks differing in their muscle growth rates during the final stages of egg incubation and the first week after hatching. Expression of the *MyoD* gene was quantified by quantitative real-time PCR (qRT-PCR). The average cross-sectional area and diameter of the fibers increased from embryonic day 21 (E21), peaking at E27, and then declining slightly 7 d after hatching. The density of the fibers decreased initially but increased after hatching in both breeds and sexes. The within-breed variation in muscle fiber-type composition was greater than the average variation between the breeds. Overall, the percentage of type I fibers increased and that of type IIb fibers decreased consistently. However, the percentage of type IIa fibers was almost constant as development proceeded in both duck breeds. The profiles of *MyoD* mRNA expression were similar in both breeds, and a significantly positive relationship was observed between the expression of *MyoD* and the percentage of type IIb fibers. This study firstly revealed the characteristics of duck muscle development and differences between the two breeds differing in growth rates. Moreover, type IIb fibers might convert to type I fibers in the lateral gastrocnemius, while *MyoD* may potentially function in controlling the muscle fiber phenotype during the secondary myogenesis of muscle development.

Keywords: duck, myofiber development, *MyoD* gene, gene expression

1. Introduction

Understanding the growth and development of skeletal muscle is one of the most important aims of animal husbandry. Meat- and egg-type duck breeds have significant genetic differences in their muscle growth rates. The Gaoyou duck, an important Chinese indigenous meat-type duck breed, is characterized by fast-growing muscle, a high bodyweight, and a high rate of double-yolk eggs. In contrast, the Jinding duck is a famous Chinese indigenous egg-type duck breed, characterized by high egg production, slow-growing muscle, and a low bodyweight (CNCAGR 2011). The differences in muscle growth between the Gaoyou and Jinding ducks provide a potentially good model for studying the mechanisms underlying differences in muscle development and phenotypes.

Muscle development is a complex process. In avian

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LI Hui-fang, E-mail: lhfxf_002@aliyun.com; SHU Jing-ting, E-mail: shujingting@163.com; Correspondence LI Hui-fang, Tel/Fax: +86-514-85599266, E-mail: lhfxf_002@aliyun.com
[*] These authors contributed equally to this study.

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species, the structure and function of skeletal muscles develop and mature during the incubation period. During this time, myoblasts proliferate, differentiate into multinucleated myotubes, and finally form mature muscle fibers, in a process generally involving myofiber hyperplasia and hypertrophy (Picard *et al*. 2002). Primary myofibers form in the initial stage of myogenesis during embryonic development, and secondary myofibers form in the second wave of myogenesis during the fetal stage and account for the majority of muscle fibers (Du *et al*. 2010). The total number of skeletal myofibers is defined by hyperplasia, which occurs predominantly during embryogenesis (cattle, sheep), but may continue postnatally (pig, rabbit) (Oksbjerg *et al*. 2004; Bérard *et al*. 2011). An increase in the total number of myofibers was shown to occur during either embryogenesis or early posthatching in the breast muscles of chickens and ducks by Halevy *et al*. (2004, 2006) and Chen *et al.* (2012), respectively.

Myofibers are the functional units of individual skeletal muscles. These muscles consist of a heterogeneous population of fibers, differing in their fiber sizes, colors, and glycogen and lipid contents, which contribute to a wide variety of functional capacities. Traditionally, skeletal myofibers can be distinguished as the red slow-twitch myofibers (type I, oxidative), the white fast-twitch myofibers (type IIb, glycolytic), and the red fast-twitch myofibers (type IIa, intermediate oxidative-glycolytic). Until now, the different isoforms of the myosin heavy chain protein have seemed to be the best markers with which to characterize muscle fiber-type diversity (Pette *et al*. 2002). Traditionally, studies in the field of muscle research have relied on histochemical classification based on staining for the acid or alkaline stability of myofibrillar ATPase (mATPase) activity (Brooke *et al*. 1970).

Unlike the mammalian fetus, whose growth is supported by the sustained provision of maternal nutrients, poultry embryos undergo development in a relatively enclosed space, and the yolk sac is the sole nutrient supply for embryonic development throughout the whole incubation period. The transition from the incubation environment to that of the newborn animal is profound and is associated with major physiological changes. Skeletal muscle, in particular, must rapidly adapt to meet the demands of locomotion and to provide postural support against gravity in the newborn animal. During this period, many genes related to muscle fiber formation, including *MyoD*, *MyoG*, and *MEF2C*, are expressed (Cao *et al*. 2006; Hennebry *et al*. 2009). Muscle regulatory factors (MRFs), which belong to a family of basic helix-loop-helix transcription factors, initiate the formation of muscle fibers and regulate the transcription of muscle-specific genes (Pas *et al*. 2007). *MRF* family members include *MyoD*, *Myf6* (MRF4/herculin), *Myf5*, and myogenin. Among the four members of the MRFs, *MyoD* is usually expressed earliest during animal muscle development (Goldhamer *et al*. 1992), and its roles at different stages of muscle development are dissimilar (Mesires and Doumit 2001). In several vertebrates, *MyoD* mRNA and protein are relatively more abundant in fast-twitch muscle (Ekmark *et al*. 2007; Ehlers *et al*. 2014; Zhu *et al*. 2014). It is widely accepted that *MyoD* directly activates the expression of many additional transcription factors, including *MyoG*, and acts in a feed-forward mechanism in cooperation with those factors to directly activate muscle genes expressed later in the differentiation program (Penn *et al*. 2004; Cao *et al*. 2006). However, the mechanisms underlying the regulation of myofiber differentiation by *MyoD* are still unclear.

The lateral gastrocnemius (LG) skeletal muscle is one of the largest muscles in the back of the leg, and contains a mixture of fiber types in the adult. There have been no reports of the histochemical characteristics in different developmental stages of the LG muscle in the duck. Furthermore, whether the expression profile of *MyoD* differs in different duck breeds is still unclear. Consequently, in the present study, we used the late-term embryos and newly hatched ducklings of the Gaoyou and Jinding duck breeds as animal models, which differ in their growth rates, and characterized the differences in the morphologies and fiber-type compositions of the LG using a mATPase-based technique. The expression profiles of the *MyoD* gene during ontogenesis were also investigated. In this study, we comprehensively analyzed the characteristics of duck muscle development, revealing changes in muscle development from the late incubation period to the early posthatching period and differences between the two breeds. We also examined the differential expression of *MyoD* in the two breeds, to provide preliminary data on the roles of *MyoD* in duck embryonic myogenesis and muscle-type differentiation.

2. Results

2.1. Growth performance in the early developmental stages of the two duck breeds

The bodyweight and leg weight measurements for the Gaoyou and Jinding duck breeds throughout the observation period are shown in Fig. 1. The bodyweights and leg weights showed similar developmental profiles in the two duck breeds. Bodyweight increased significantly (P<0.05) from embryonic day 21 (E21) to E25, increased slightly from E25 to E27, and then increased significantly (P<0.05) by 7 d posthatching (7 d PH). Leg weight increased significantly (P<0.05) during late-embryonic and posthatching development. In total, the rate of leg weight increase was higher than that of bodyweight increase. Both the bodyweights and leg