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Changes in relative organ weights and intestinal transporter gene expression in embryos from white Plymouth Rock and WENS Yellow Feather Chickens

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ABSTRACT

This study was conducted to evaluate the embryonic development of broilers with different growth rates and correlate the differences between the amino acid transporter and peptide transporter gene expression patterns to the growth of the small intestine. The results showed that the body and yolk weights of the White Plymouth Rock (WPR) embryos were higher than those of the WENS Yellow Feather Chicken (WYFC) embryos although the relative embryonic body weights were inversely correlated. We studied nine organs and classified them into four clusters according to their changes in relative weight during the hatching process. The levels of gene expression of SLC7A9, SLC1A1 and SLC15A1 in the small intestine increased during embryo development and were affected by breed. Breed-specific differences in embryonic development were observed for SLC7A9, SLC1A1 and SLC15A1 gene expression. When represented as a function of SLC7A9, SLC1A1 or SLC15A1 gene expression, strong correlations were observed for the weight of small intestine. We conclude that WPR embryos have a higher absolute growth rate but a lower relative growth rate in comparison with WYFC embryos. Moreover, the expression levels of the SLC7A9, SLC1A1 or SLC15A1 genes can be used as indicators for the growth of the small intestine.

1. Introduction

The embryonic growth rate is critical for postnatal growth (Al-Murrani, 1978). Embryo development is affected by the age of the breeder, egg size, egg storage and incubation conditions and genotype (Azzam and Mortola, 2007; Everaert et al., 2008; Mortola and Al Awam, 2010). Moreover, growth of the same organ in different breeds and growth of different organs in the same breed follow different developmental trajectories (Azzam and Mortola, 2007; Lindgren and Altimiras, 2011). In previous studies, many physiological indicators of chick development have been identified (Tona et al., 2003, 2004; Everaert et al., 2008; Tona et al., 2010). The resonant frequency of the egg is used as an indicator of embryonic development because it measures embryonic fluid formation (Bamelis et al., 2002). The expression of intestinal amino acid or peptide transporter genes is also correlated with chick growth (Humphrey et al., 2004; Gilbert et al., 2007). Dietary protein is broken down into small peptides (di- and tri-peptides) and free amino acids, which are taken up by specific transporters located on the brush border membrane of enterocytes. Peptides and amino acids are delivered to tissues through the blood to serve as precursors for a variety of bioactive molecular and energy metabolites (Bröer, 2008). Chick embryos obtain nutrients from the yolk-sac membrane, but the expression of intestinal transporters during the last few days of incubation allows the chick embryo to extract nutrients from material passing through the intestine (Speier et al., 2012). When the yolk sac is internalized into the body cavity and amniotic fluid is swallowed, transporter function at the brush border membrane becomes important for luminal nutrient absorption (Moran, 2007). The development of the chick embryo depends on the proper function of nutrient transporters. However, our understanding of developmental indicators in the chick embryo and small intestine remains somewhat limited. We hypothesize that several candidate genes expressed in the small intestine can be used as indicators of small intestine growth.

Our previous studies found that White Plymouth Rock (WPR) chickens have a larger egg size, higher hatchling and adult weight, growth rate and meat yield than those of WENS Yellow Feather Chickens (WYFC) (Wang et al., 2012a,b). The genetic background made it suitable to use WPR and WYFC to identify and investigate the differences between genetic indicators in embryonic development. Our former studies showed that the expression of SLC7A9, SLC1A1 and SLC15A1 in the small intestine showed strong correlations with embryo development (Li et al., 2011; Zeng et al., 2011). Therefore, the objective of this study was to explore: 1) the developmental differences between WPR and WYFC embryos and organs and 2) the levels of amino acid or peptide transporter gene expression as indicators of development in the small intestine in chick embryos.

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Table 1
Primers used for real-time PCR.[a]

Gene	GenBank ID	Primers	PCR product (bp)
SLC7A9	XM_414130	S: 5′-GGAACCCCAATCTATGAACC-3′ AS: 5′-GGACCCACAGCTCCAACA-3′	135
SLC1A1	XM_424930	S: 5′-GAAGTTGAGGACTGGGAAAT-3′ AS: 5′-ATGAGGGCTGTCAGAAGTG-3′	173
SLC15A1	NM_204365.1	S: 5′- ACGCATACTGTCACCATCA -3′ AS: 5′-AAAAGTCGTGTCACCCATA -3′	156
GAPDH	NM_204305	S: 5′-AGAACATCATCCCAGCGTCC-3′ AS: 5′-CGGCAGGTCAGGTCAACAAC-3′	133
β-actin	NM_205518.11	S: 5′-ATTGTCCACCGCAAATGCTTC-3′ AS: 5′-AAATAAAGCCATGCCAATCTCGTC-3′	113
HPRT1	NM_204848.17	S: 5′-TTGTTGGATACGCCCTCGACTAC-3′ AS: 5′-CCATAGCACTTCAACTGTGCTTTCA-3′	117
TBP	NM_205103	S: 5′-CACCTCTGTACCCGTCCC-3′ AS: 5′-GCAACCAAGATTCACCGT-3′	122
RPL13	NM_204999.1	S: 5′-GCCCGACTGTCAGATACCACAA-3′ AS: 5′-GAGATTCCAATCGTCCGAGCA-3′	109
α-tubulin	NM_001080860	S: 5′-GAGAACACGGATGAAACCTACT-3′ AS: 5′-TGGTGGCTGACACTAAATGG-3′	118

[a] SLC7A9 = solute carrier family 7 member 9; SLC1A1 = solute carrier family 1 member 1; SLC15A1 = solute carrier family 15 member 1; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; HPRT1 = hypoxanthine phosphoribosyltransferase 1; TBP = TATA box binding protein; RPL13 = ribosomal protein L13.

2. Materials and methods

2.1. Experimental design

A total of 144 hatching eggs that were provided by WENS Co., Ltd. (Yunfu, China) were used for this study. A commercial flock of White Plymouth Rock (WPR) chickens (*Gallus gallus*) produced 72 eggs; the other 72 eggs were produced by an indigenous breed of broilers called WENS Yellow Feather Chickens (WYFC) (*G. gallus*), with a marketing body mass that is approximately 1.5 kg for 105-day-old females. Both flocks were 30 weeks old and fed with the same diet. The fresh egg masses were 44.17 ± 0.30 g and 58.40 ± 0.23 g for WYFC and WPR, respectively ($P<0.0001$). Each of the 72 eggs from the WPR or WYFC flocks were weighed, numbered and divided equally into 6 groups based on similar egg weights without statistical significance ($P>0.90$) before hatching. A total of 12 groups of fertilized eggs were incubated at 37.8 °C with a relative humidity of 60% and were turned 90° every 2 h until they reached embryonic day 18. They were transferred to the same hatchery for the last 3 days of incubation. All of the animal procedures were performed according to protocols that were approved by the Animal Care Committee of South China Agricultural University (Guangzhou).

2.2. Measurement of embryonic body and organ weights

Embryonic development was evaluated by the absolute and relative weights of the body, yolk and organs. Twelve eggs/hatchlings per breed were sampled on embryonic days (E) 10, 12, 14, 17, 19 and the day of hatching (DOH). The animals were killed using cervical dislocation on measurement days. The eggs were opened and the yolks and bodies were dissected and drained of blood and other fluids. They were weighed on a digital balance accurate to 10^{-4} g. The head, thorax (with both meat and bone), leg (shank and thigh of the left leg with both meat and bone), heart, liver, lungs, kidneys, stomach and small intestine were isolated and weighed. The stomach and small intestines were exenterated before they were weighed. The relative weights of the body and yolk of each embryo were normalized to the fresh egg weight, and the relative weights of the organs were normalized to the body weight without the yolk.

2.3. RNA extraction and real-time RT-PCR

The small intestines, which were sampled on E10, E14, E17 and DOH, were frozen in liquid nitrogen and ground using a mortar and a pestle. The total RNA was isolated from small intestine powder using the TRIZOL reagent (Invitrogen, Carlsbad, USA) and treated with DNase I according to the manufacturer's instructions (TaKaRa, Shiga, Japan). We checked the integrity of the 18S- and 28S-ribosomal RNA bands and confirmed the RNA quality by the absence of genomic DNA. The optical density of the RNA was measured using a NanoDrop 2000 UV–Vis spectrophotometer (Thermo, Wilmington, MA, USA) and the 260 nm/280 nm ratios ranged from 1.80 to 2.00. After the RNase-free DNase treatment, the RNA (2 μg) was reverse-transcribed to cDNA in a reaction with oligo(dT)18 primers (Sangon, Shanghai, China), M-MLV reverse transcriptase (Promega, Madison, WI, USA), M-MLV

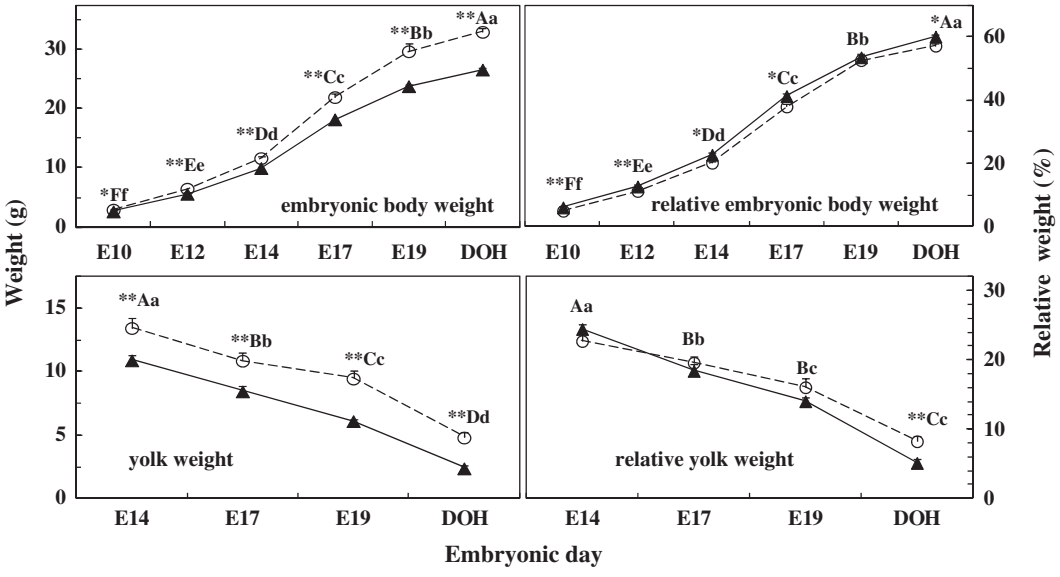

Fig. 1. Mass (g) and relative weight (absolute weight normalized to fresh egg weight, %) of embryonic body and yolk for White Plymouth Rocks (open circles) and WENS Yellow Feather Chickens (filled triangles). n = 12. Values are presented as mean ± SEM. *Statistically significant difference between the two groups ($P<0.05$). **Statistically highly significant difference between the two groups ($P<0.01$). A–F, means without a difference among embryonic days for White Plymouth Rocks ($P<0.05$). a–f, means without a difference among embryonic days for WENS Yellow Feather Chickens ($P<0.05$).