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Hypoxic Adaptation and Myoglobin Expression in Heart Tissue of Tibet Chicken Embryo

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Abstract: Myoglobin (Mb) is a classical member of the globin family and plays an important role in the oxygen transportation or storage. As a unique native chicken breed in high altitude, Tibet chicken has the good adaptation to hypoxia. Here we present the first detailed analysis of Mb expression on hypoxic adaptability in chicken heart. In the present study, fertile eggs of Tibet chicken, Shouguang chicken and Silky chicken were exposed to sustained hypoxia (13% O_2) and normoxia (21% O_2). Chicken embryo hearts were collected on days 16 and 20 of incubation to examine the effect of hypoxia on Mb. The results showed that there is no significant difference in content or expression levels of Mb among the three chicken breeds in the normoxic environment. Tibet chicken embryos had the heaviest heart weight among these chicken breeds and the Mb content of all chicken breeds had increased in hypoxia comparing with that in normoxia. Under the hypoxic environment, Mb mRNA levels of Tibet chicken were lower than that of Shouguang and Silky chicken, indicating that the hypoxic degree between Tibet chicken and other lowland chicken was different. The smaller changes of Mb content and expression levels in hypoxia in Tibet chicken embryo suggests Tibet chicken had the better hypoxic adaptation ability in utilizing oxygen in heart tissue. The results showed that Mb is up-regulated by hypoxia and may play an important role in mediating heart hypoxic adaptation.

Key words: Myoglobin, Tibet chicken embryo, hypoxic adaptation, Mb content, eggs

INTRODUCTION

Myoglobin, a protein with a rich history is a cytoplasmic hemoprotein, developed in red muscle in response to mitochondrial demand for oxygen (Wittenberg, 1970) and transports oxygen from the sarcolemma to the mitochondria of vertebrate heart and red muscle cells (Wittenberg and Wittenberg, 1989; Takahashi and Doi, 1998; Wittenberg and Wittenberg, 2003), which reversibly binds O_2 by its heme residue (Ordway and Garry, 2004). In the recent decade, gene disruption technologies have been utilized to study the functions of Mb under the hypoxic stress (Garry *et al*., 1998; Meeson *et al*., 2001; Schlieper *et al*., 2004).

Hypoxia is the main physiological challenge which threatens the survival of organisms in high-altitude areas. Many researchers found that Mb content and expression [redacted]

Mb expression in nonmuscle tissues. In the Tibetan plateau, Tibet chicken has developed subtle mechanism of surviving in a hypoxic environment in the long-term adaptation, demonstrated that the adaptability of Tibet chicken embryos to hypoxia is significantly higher than other lowland chicken breeds (Gou *et al*., 2005; Wang *et al*., 2007; Bao *et al*., 2007; Zhang *et al*., 2008; Li and Zhao, 2009). Until now, our group had confirmed that the embryo death was mainly observed between Tibet chicken and the other lowland chicken when the egg was incubated at days 18-21, showing that the embryos at late phase of incubation were most sensitive to hypoxia (Bao, 2007; Gou *et al*., 2007; Zhang *et al*., 2007). Therefore, we supposed that the different levels of Mb content and mRNA expression at days 16 and 20 in the heart under the hypoxic condition may be helpful for Tibet chicken to adapt the environment. After examining the [redacted]

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Table 1: Primer sequence and size of Real-time PCR analysis

Target gene	GenBank No.	Sense/Antisense	Size (bp)
Mb	XM_416292	F: 5' GAAAAGTGGAGGCCGACAT 3'	141
		R: 5' TCAGATCTTCAGAGCCCTTCA 3'	
28s rRNA	M59792	F: 5'-GGAGCCCCGGGGAGAGTTC-3'	140
		R: 5'-GGATTTTCACGGGCCAGCGAGAG-3'	
Hprt	AJ132697	F: 5'- CAACCTTGACTGGAAAGAATGT-3'	171
		R: 5'-CAACAAAGTCTGGCCGATAT-3'	

MATERIALS AND METHODS

Real-time PCR: cDNA PCR was performed using the SYBR Green master mix (ABI Applied Biosystems, US)

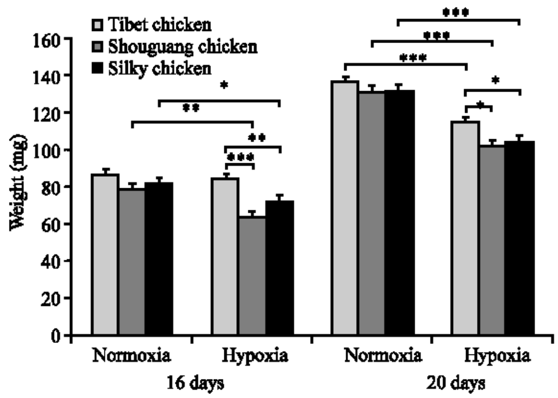


Fig. 1: Weight of hearts in different chicken breeds under hypoxic and normoxic conditions. Each bar represents the mean±SE for each group and Tibet chicken contains 16 samples and Shouguang chicken and Silky chicken contain 10 samples, respectively. *Significant difference at 5% level, **Significant difference at 1% level, ***Significant difference at 0.1% level

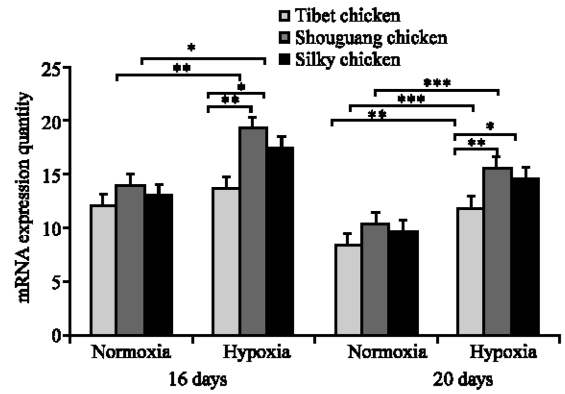
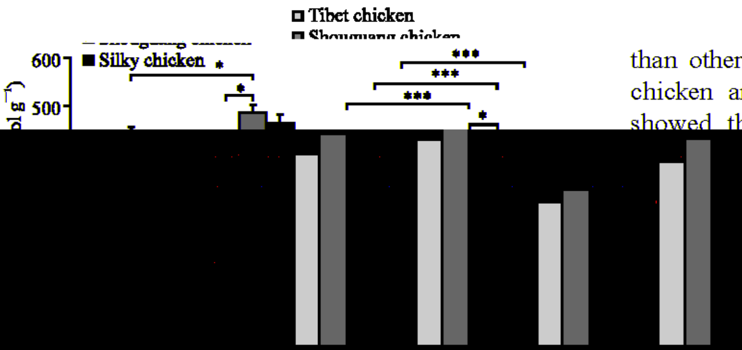


Fig. 3: Mb mRNA expression in different chicken breeds under hypoxic and normoxic conditions. Each bar represents the mean±SE for each group and each group contains 10 samples for mRNA expression level

Mb mRNA expression levels: As shown in Fig. 3, no significant difference on the interactions of breed x oxygen concentration was discovered. The expressions of Mb mRNA in Tibet chicken on two stages were lower than other chicken breeds in hypoxia (p<0.05 in Silky chicken and p<0.01 in Shouguang chicken). Results showed that Mb mRNA expressions were higher in [redacted]



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In the present study, the results firstly showed the

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