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Expression Analysis of *HIF-1α* and *HIF-2α* Genes in Tibetan Chicken under Normoxia and Hypoxia

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Abstract: Tibetan chicken is one of those chicken breeds that could adapt to high altitude and low O_2 tension environment; it owes an integrative genetic mechanism for hypoxia adaptability compared to lowland chicken breeds. HIF-1α and HIF-2α are the central factors playing important roles in maintaining organisms's oxygen homeostasis. In this study, the expression of *HIF-1α* and *HIF-2α* genes were investigated by Real Time-PCR in Tibetan chicken and Dwarf chicken embryo brain tissue under normoxia (21% oxygen concentration) and hypoxia (13% oxygen concentration). The results showed that Tibetan chicken always had lower mortality than Dwarf chicken during the incubation. We also detected higher HIF-αs expression at the late of incubation (18th day) compared with that at 12th day in all tested samples; we further obtained the expression pattern of *HIF-αs* genes in dead embryos was opposite to that in live embryos.

Key words: Tibetan chicken, HIF-1α, HIF-2α, normoxia, hypoxia, adaptability

INTRODUCTION

Hypoxia Inducible Factor-1α (HIF-1α) and Hypoxia Inducible Factor-2α (HIF-1α) were the central factors in keeping oxygen homeostasis of organisms, their function were regulated by O_2 concentration strictly (Uchida *et al*., 2004; Weidemann and Johnson, 2008). The regulation is done through hydroxylation at protein positions of Pro402, Pro564 and Asn803 which were catalyzed by PHD2 (Prolyl Hydroxylase Domain Protein 2, PHD2) and FIH-1(Factor Inhibiting HIF1, FIH-1). However, above regulation is O_2 dependent and it couldn't catalyze the reactions under hypoxia or anaerobia. Subsequently, HIF-αs could be stabilized and transported into nuclear to form HIF-1 and HIF-2 by dimerizing with HIF-1β which is another Hypoxia inducible factor under hypoxia; finally HIF-1 and HIF-2 could regulate their target genes to adapt to hypoxia by binding the dimerizing complex to the HREs (Hypoxia Response Element, HRE, usually 5'RCGTG3') of target genes which always exist in the promoter or enhancer region of targeted genes. *HIF-αs*' target genes cover EPO (Erythropoietin, EPO), VEGFA (Vascular Endothelial Growth Factor A, VEGFA) and several genes encode glycolytic enzymes such as PGK (Phosphoglyceate Kinase, PGK), PDK-1 (Pyruvate Dehydrogenase Kinase-1, PDK-1) (Kim *et al*., 2006; Papandreou *et al*., 2006; Semenza, 2007).

Tibetan chicken has been living in the environment of high altitude and low-O_2 tension for many centuries and owned an integrative genetic mechanism for its adaptation to hypoxia. However, Dwarf chicken is a lowland chicken which should be very sensitive to hypoxia and have to bear the serious hypoxic stress when exposures to hypoxia. In the present study, the expression pattern of *HIF-αs* genes were investigated in both Tibetan chicken and Dwarf chicken, the purpose of this study is to provide some clues for better understanding the adaptive mechanism of Tibetan chicken under hypoxia.

MATERIALS AND METHODS

Samples collection: Fertalized eggs of Tibetan chicken and Dwarf chicken were hatched in incubator under normoxia (21% O_2 concentrition) and hypoxia (13% O_2 concentrition), respectively. Three dead and six live embryonic brains were taken from different breeds at 8, 12 and 18th days of incubation stage under above two different treatments. The samples were stored in liquid nitrogen until use.

The expression of HIF-αs mRNA: RNA was extracted in TRNzol and reverse transcribed into cDNA. Real Time-PCR was performed with the SYBR Green reagent using the equipment of BIO-RAD Real Time-PCR9600 (BIO-RAD). Special primers designed by prime primer 5.0 were as follows: GAPDH as a reference gene (F5'-ATAC ACAGAGGACCAGGTTG-3', R: 5'-AAACTCATTGT CATACCAGG-3', product length is 130 bp); HIF-1α (F: 5'-

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AGTTCACCTGAGCCCAGTA-3', R: 5'-AGGAGCCAA CATTTCCAAG-3', product length is 169 bp) and HIF-2α (F: 5'-CCTACAGCCTCAGTGTATCAT-3', R: 5'-ATCA CTCTTGTCCCTCCCT-3', product length is 163 bp).

Data analysis: The collected raw data were standardized by BIO-RAD CFX Manager Version 1.5 (BIO-RAD) software after that the standardized data was analysis completed by SAS 9.1 in Duncan's multiple range test.

RESULTS AND DISCUSSION

In the present study, the hatchability was different between the two experimental chicken breeds. The data showed that Tibetan chicken was 95.55% under normoxia and 74.55% under hypoxia and that of dwarf chicken was 89.68% under normoxia and 15.03% under hypoxia. We also found that the mortality of the two breeds under normoxia was higher than that under hypoxia ($p<0.01$).

Under normoxia, the data of *HIF-αs* genes indicated that Tibetan chicken had higher HIF-αs expression at 18th day than that of Dwarf chicken ($p<0.01$, Table 1 and Fig. 1) while we didn't find significant differences between them at other sampling time. There was higher HIF-αs mRNA level at 18th day than that at 12th and 8th day within Tibetan chicken breed ($p<0.01$, Table 1 and Fig. 1) however, there was no expression difference found within Dwarf chicken breed ($p>0.05$, Table 1 and Fig. 1).

Under hypoxia, there was no significant difference of HIF-αs mRNA expression between Tibetan and Dwarf chicken at the same incubation stage ($p>0.05$, Table 1 and Fig. 1). But there was higher expression of *HIF-αs* genes at 18th day than that at 12th day in both Tibetan and Dwarf chicken breeds ($p<0.01$, Table 1 and Fig. 1); we also found that there was higher HIF-2α expression level at 8th day than that 12th day within Dwarf chicken ($p<0.05$, Table 1 and Fig. 1); we further obtained the higher expression of HIF-αs in dead embryos than that in live ones at all incubation stages in both Tibetan and Dwarf chicken breeds (Table 2 and Fig. 2). It is very interesting that the expression pattern of *HIF-αs* genes in dead embryos is opposite to that in live embryos (Table 2 and Fig. 2).

Previous studies have shown that there are two key stages during the chicken embryo development (Kuurman *et al*., 2001). One is the beginning of incubation, another one is the late of incubation and both of them are related to respiration type transformation. During this period, fast cell growth and transformation of respiration type could affect the tender embryos by producing hypoxia or other stress factors (Hamburger and Hamilton, 1951). In the study, we detected higher HIF-αs expression at the late of incubation (18th day) compared with that at 12th day in all tested samples; no matter they are under normoxia or hypoxia situation. Above results might be caused by chicken's respiration type changed from allantois respiration to lung respiration during the incubation days 15-20th and this biological turning point could produce hypoxia stress. As we already know that hypoxia prevent HIF-αs from hydroxylation by the PHD and FIH-1 which allow the accumulation of HIF-αs products. We also found higher HIF-2α expression at 18th day in Tibetan chicken than that in Dwarf chicken under normoxia which might suggest that Tibetan chicken has stronger endurability when encountered the hypoxia stress. On the other hand, this result might suggest the

Table 1: Expression level of the *HIF-αs* gene in Tibetan chicken and Dwarf chicken (Mean±SE)

		Treatments					
		Normoxia			Hypoxia		
Genes	Incubation stage	8th day	12th day	18th day	8th day	12th day	18th day
HIF-1α	Tibetan chicken	0.68±0.10*	0.58±0.07	1.70±0.35^{a}**	1.11±0.24*	0.48±0.08	1.80±0.27**
	Dwarf chicken	0.41±0.02*	0.47±0.11*	0.60±0.11^{b}*	1.82±0.26**	0.34±0.05*	1.87±0.62**
HIF-2α	Tibetan chicken	0.15±0.03	0.21±0.03	0.75±0.15^{a}**	0.23±0.05	0.22±0.05	1.02±0.22**
	Dwarf chicken	0.12±0.01	0.17±0.02	0.37±0.05^{b}	0.43±0.12*	0.12±0.04	0.76±0.30**

^{a,b}Means of the same data within the same column are extremely ($p<0.01$) different; *Means of the same data within the same row are significantly ($p<0.05$) different and **are extremely ($p<0.01$) differently

Table 2: Expression level of the *HIF-αs* gene in dead Tibetan chicken and Dwarf chicken embryoes under hypoxia (Mean±SE)

	HIF-1α			HIF-2α		
Incubation stage	8th day	12th day	18th day	8th day	12th day	18th day
Live Tibetan chicken	0.78±0.17	0.33±0.05	1.25±0.19	0.40±0.09	0.38±0.08	1.76±0.37
Dead Tibetan chicken	4.01±0.60**	3.05±0.37**	2.72±0.83*	4.39±1.15**	5.29±1.08**	3.97±1.15*
Live Dwarf chicken	1.08±0.16	0.19±0.03	1.11±0.38	0.43±0.12	0.12±0.04	0.76±0.30
Dead Dwarf chicken	13.63±2.94**	7.68±1.41**	1.42±0.35	7.39±0.28**	2.54±0.47**	1.49±0.05

*Means of the same variable within the same column are significantly ($p<0.05$) different and **are extremely ($p<0.01$) differently

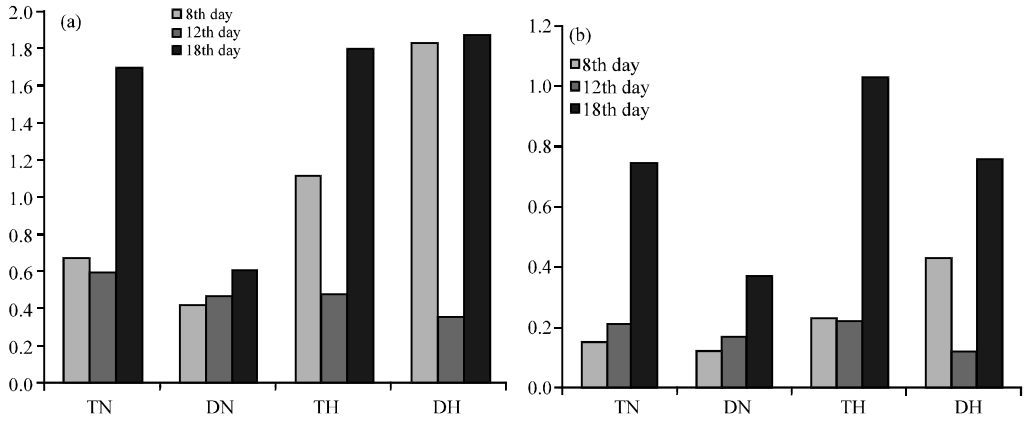

Fig. 1: a) Expression of TC and DC *HIF-1α* gene in survival embryos under normoxia and hypoxia at different stages; b) Expression of TC and DC *HIF-2α* gene in survival embryos under normoxia and hypoxia at different stages; TN = Tibetan chicken under normoxia; TH = Tibetan chicken under hypoxia; DN = Dwarf chicken under normoxia; DH = Dwarf chicken under hypoxia; TC = Tibetan Chicken; DC = Dwarf Chicken

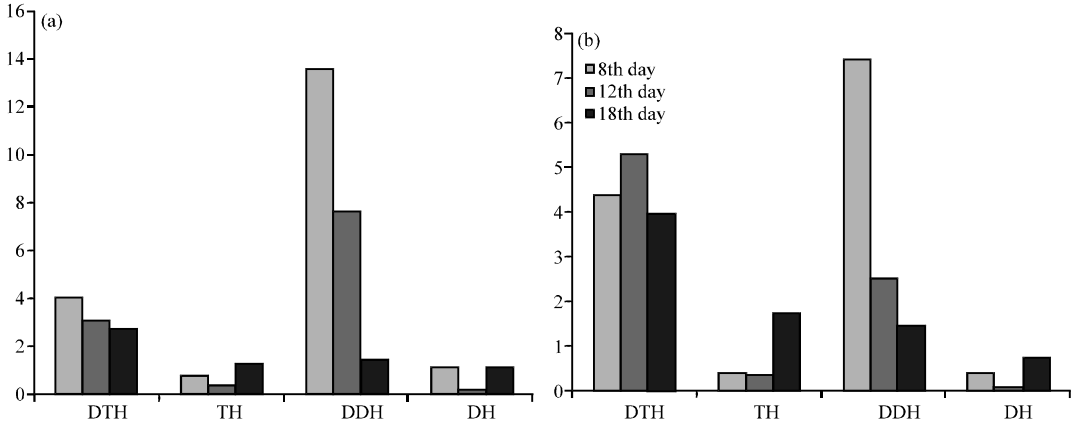

Fig. 2: a) Expression of *HIF-1α* gene in survival and dead embryos under hypoxia and normoxia at different chicken groups; b) Expression of *HIF-2α* gene in survival and dead embryos under hypoxia and normoxia at different chicken groups. DTH = Dead Tibetan chicken under hypoxia; DDH = Dead Dwarf chicken under hypoxia

higher expression of *HIF-αs* genes will increase the adaptation of chicken embryo to hypoxia (Tibetan chicken's hatchability: 74.55%) because chicken embryo bears hypoxia stress at the 18th day of incubation even though it was under normoxia. The opposite expression pattern was found between live and dead embryos which further confirms higher expression of *HIF-αs* genes is an indicator for better adaptation to hypoxia. However, there was no significant difference in HIF-αs expression at 8, 12 and 18th day between Tibetan and Dwarf chicken under hypoxia. We assumed that the survival embryos of these two breeds were both able to adapt to hypoxia and they share similar HIF-αs expression pattern.

This is the first report on expression of *HIF-1α* and *HIF-2α* genes in Tibetan chicken by investigating dead and survival chicken embryos in one experiment. It confirms the important roles of *HIF-αs* genes in chicken's adaptation to hypoxia. However, further studies focused on the target genes of HIF-αs should be designed to reveal the molecular mechanism of Tibetan chicken hypoxia adaptability.

CONCLUSION

The study indicated Tibetan chicken's hypoxia adaptability was stronger than that of Dwarf chicken when exposured to hypoxia stress. This study provides clues for better understanding the hypoxia adaptation of Tibetan chicken.

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