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Effects of Hypoxia on Activities of GPx, GSR and GST in Tibet Chicken and Silky Chicken Hearts

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Abstract: This study was performed to investigate whether differences exist in activities of Glutathione Peroxidase (GPx), Glutathione Reductase (GSR) and Glutathione S-transferase (GST) in hearts between Tibet chicken and a lowland chicken breed (Silky chicken). At the end of 5 days of age, 24 chicklings of each breed were divided into 3 groups treated with three different oxygen concentrations, respectively for 20 h. Activities of the three enzymes in chicken hearts were determined spectrophotometrically.

Key words: Tibet chicken, silky chicken, glutathione peroxidase, glutathione reductase, glutathione S-transferase, hypoxia

INTRODUCTION

Reduced glutathione (GSH) is an important intracellular thiol taking part in several cellular functions including antioxidant defences, detoxification of xenobiotic chemicals or endogenous toxic substances such as lipid hydroperoxide (Rushmore and Pickett, 1993; Singh *et al*., 2001; Wang, 2005; Yang *et al*., 2006). Oxidized glutathione (GSSG) also known as glutathione disulfide is the dimeric form of glutathione. As an important antioxidant, the functions of glutathione are mainly catalyzed by the three enzymes of Glutathione Peroxidase (GPx), Glutathione Reductase (GSR) and glutathione S-transferase (GST) (Meister, 1988; Wu *et al*., 2004). Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) and various hydroperoxides (ROOH) to water or the corresponding alcohols (ROH) using GSH as reducing substrate as shown in Eq. 1 and 2 (Wang, 2005).

$$2GSH+H_2O_2 \xrightarrow{GPx} GSSG+2H_2O \quad (1)$$

$$2GSH+ROOH \xrightarrow{GPx} GSSG+ROH+H_2O \quad (2)$$

Glutathione reductase catalyzed the conversion of GSSG to GSH in the present of reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as shown in Eq. 3 (Rall and Lehninger, 1952).

$$GSSG+NADPH+H^+ \xrightarrow{GSR} 2GSH+NADP^+ \quad (3)$$

Glutathione S-transferase catalyzes the necleophilic addition of GSH to various electrophiles including xenobiotic chemicals and endogenous toxic substances such as lipid hydroperoxide and thereby defends cells from toxic effects of these compounds (Rushmore and Pickett, 1993; Singh *et al*., 2001; Yang *et al*., 2006).

Hypoxia is a deleterious environmental factor which can increase the production of Reactive Oxygen Species (ROS) (Duranteau *et al*., 1998) and oxidative stress obviously (Askew, 2002).

Tibet chicken originates from high-altitude area and adapts itself well to hypoxia and Silky chicken is a lowland chicken breed raised and bred in lowland area. The previous study, using chicken livers as experimental materials, showed that Tibet chicken and Silky chicken were identical in activities of GPx and GST but not in GSR activity when these birds were under normoxia condition or extreme hypoxia condition (Bao *et al*., 2011). In view of the important role of heart in life, it is essential to study the differences of the heart responses to hypoxia between Tibet chicken and the lowland chicken which may enrich the understanding of the mechanism of adaptation to hypoxia in chicken. In the present study, efforts were made to investigate whether there were any differences in activities of the three antioxidant enzymes in the hearts of Tibet chicken and Silky chicken under normoxia or hypoxia conditions.

MATERIALS AND METHODS

Experimental design are carried out according to the scheme described by Bao *et al*. (2011) as follows: 24

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chicklings of Tibet chicken and equivalent ones of Silky chicken were raised in normoxia condition and fed a commercial diet with 20% CP, 11.92 MJ kg^{-1} of gross energy and 0.77% of cystine and methionine; feed and water were provided ad libitum; at the end of 5 days of age, birds of each kind were divided into 3 groups of 8 each and two of the there groups of each breed were placed in normoxia condition (21% of oxygen concentration, 21% O_2) and moderate hypoxia condition (14% O_2), respectively for 20 h; another group of each breed was put in 14% O_2 for 14 h and then in 10.5% O_2 for 6 h (14/10.5% O_2).

All birds were killed by cervical dislocation immediately after the end of the hypoxia exposures and the hearts were excised rapidly. After blood, fat and connective tissue were removed, the hearts were put into 2 mL microcentrifuge tubes with ice-cold SETH buffer (250 mmol L^{-1} sucrose, 2 mmol L^{-1} EDTA, 10 mmol L^{-1} Tris, 5×10^4 IU L^{-1} heparin, pH 7.4) in an ice bath and finely minced immediately then the sample was carefully homogenized with a tissue grinder kept in an ice bath and frozen in 100 μL aliquots in liquid nitrogen rapidly and then stored at -80°C for enzyme estimations.

Homogenate protein was estimated by the Bradford method using Bovine Serum Albumin (BSA) as standard. Activities of GPx, GST and GSR were determined spectrophotometrically according to Bao *et al*. (2011). Data analysis were performed using t-tests of Excel.xp (Microsoft Corp.) or Duncan test of SPSS 13.0 (SPSS Inc.).

RESULTS AND DISCUSSION

Activities of Gpx, GSR and GST in hearts of Tibet chicken and Silky chicken are shown in Table 1. There was no difference in the activity of GPx between Tibet chicken and Silky chicken in 14% O_2 or 14%/10.5% O_2 while under the normoxia condition (21% O_2), the Gpx activity of Tibet chicken was higher ($p = 0.014$) than that of Silky chicken. In the case of GPx activity of Tibet chicken, there was a difference ($p = 0.016$) between the two groups in 14% O_2 and 14%/10.5% O_2, respectively. And no difference was found when Tibet chicken was housed in normoxia compared with in 14% O_2 ($p = 0.083$) or 14%/10.5% O_2 ($p = 0.162$). In the case of GPx activity of Silky chicken, values were decreased with the decline of oxygen concentration and there was a difference ($p = 0.018$) between the two groups of Silky chicken in 21% O_2 and 14%/10.5% O_2 and no statistically significant difference was found when Silky chicken was treated with 14% O_2 compared with 21% O_2 ($p = 0.054$) or 14/10.5% O_2 ($p = 0.256$). The activity of GSR of Tibet chicken was always higher in value than that of Silky chicken in the present study. There were differences in the activity of GSR between Tibet chicken and Silky chicken when they were treated with 14/10.5% O_2 ($p = 0.001$) and 21% O_2 ($p = 0.049$) together but no statistically significant difference was found when they were exposed to 14% O_2 for 20 h ($p = 0.183$). There was no statistically significant change in GSR activity among the three groups of Tibet chicken whereas there was a decrease in the enzyme activity of Silky chicken when birds was treated with 14/10.5% O_2 compared with 21% O_2 ($p = 0.012$) or 14% O_2 ($p = 0.011$). And no difference was found in GSR activity between the two groups of Silky chicken treated with 21% O_2 and 14% O_2 ($p = 0.13$). There was no difference in the activity of GST between Tibet chicken and Silky chicken under the same oxygen concentrations in the present study. The moderate hypoxia (14% O_2) increased ($p<0.05$) the GST activity of each breed but no difference in the enzyme activity was found between the two groups of each breed in 14% O_2 and 14/10.5% O_2.

Reduced glutathione is an abundant antioxidant in cells and the [GSH]:[GSSG] ratio often seems to be a sensitive indictor of oxidative stress (Kidd, 1997). Glutathione peroxidase is the main antioxidant enzyme which has a pivotal role in cell antioxidant protection (Guerin *et al*., 2001) but it requires GSSG to be reduced to GSH by GSR to maintain the level of the [GSH]:[GSSG] ratio. The reduction of GSSG to GSH catalyzed by GSR plays an important role in maintaining GSH level in cells. The higher GSR activity in Tibet chicken compared with Silky chicken in the present study, agreed with the result of the previous study with chicken liver (Bao *et al*., 2011), implies the stronger ability of Tibet chicken than that of Silky chicken to maintain GSH level and against the oxidative stress caused by hypoxia. This study enriched the understanding of the differences between the highland chicken and the lowland chicken and the molecular mechanism of these differences needs to be further studied.

Table 1: Activities of GPx, GSR and GST in Tibet chicken and Silky chicken hearts[1] (n = 8)

	GPx (units mg^{-1} of protein)		GSR (units g^{-1} of protein)		GST (units mg^{-1} of protein)	
Oxygen treatments	Tibet chicken	Silky chicken	Tibet chicken	Silky chicken	Tibet chicken	Silky chicken
21% O_2	207.19±24.65^{ab}*	309.87±26.84^{a}	266.71±18.43*	227.90±11.79^{a}	18.26±1.09^{b}	20.05±1.00^{b}
14% O_2	256.97± 23.62^{a}	236.34±33.36^{ab}	289.656±21.54	259.53±23.97^{a}	25.89±1.23^{a}	25.49±1.88^{a}
14%/10.5% O_2	168.94± 27.75^{b}	201.43±39.76^{b}	294.87±11.9**	180.08±14.53^{b}	27.07±1.93^{a}	24.63±2.20^{a}

^{a,b}Values of the same breed within a column with different letters are different ($p<0.05$); *Values of the same variable within the same row are different ($p<0.05$); **Values of the same variable within the same row are different ($p<0.01$); [1]Values represent the mean±SE

CONCLUSION

The results showed that the activity of GSR in heart of Tibet chicken was always higher in value than that of Silky chicken in the present study which may imply that Tibet chicken was stronger than Silky chicken to maintain GSH level and counteract the oxidative stress in hypoxia conditions.

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