The Journal of Poultry Science, 45: 180–185, 2008
http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.45.180

Effect of Breed, Heterosis and Sex on Body Weight, and Glutathione Peroxidase Activities in Red Blood Cell Haemolysate and Blood Plasma at the Age of Sexual Maturity in Chickens

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The purpose of the present study was to collect information about the possible genetic background of glutathione peroxidase (GSHPx) activity in four pure breeds and their crosses of chickens, and its correlation with body weight (BW) and studying the effect of cross-breeding and sex on GSHPx activity. Blood samples were collected from six breeds of chickens, New Hampshire (NH), Naked Neck Plymouth (NNP) and their cross (NH×NNP), White Plymouth Rock (WPR), Naked Neck New Hampshire (NNNH) and their cross (WPR×NNNH) at the age of sexual maturity. GSHPx activity measured in blood plasma (BP) and red blood cell haemolysate (RBC). Results showed that there were significant differences in BW and GSHPx activities in RBC and BP among genetic groups and their crosses. NNP had the highest average BW (1.97 kg) and NH had the lowest (1.11 kg). WPR had the highest RBC GSHPx activity (6.24 U/g protein) and NH×NNP cross had the lowest (3.98 U/g protein). WPR×NNNH had the highest BP GSHPx activity (7.23 U/g protein) and NNNH had the lowest (6.22 U/g protein). Crosses had intermediate values for BW compared to their parents. The two crosses had lower RBC GSHPx activity than their parent breeds. NH×NNP had lower BP GSHPx activity than their parent breeds while WPR×NNNH had higher values. Sex had significant effect on BW and GSHPx activity in RBC and BP, males had higher values than females for BW and BP GSHPx activity while the opposite was found in RBC GSHPx activity. Heterosis as a percentage of the midparent values of the GSHPx activities in RBC and BP also average BW has high values and affected by sex. Significant negative correlation was found between BW and RBC GSHPx activity (-0.27; $P<0.01$) while, positive correlation was found between BW and BP GSHPx activity.

Key words: body weight, breed, glutathione peroxidase, heterosis, sex

J. Poult. Sci., 45: 180–185, 2008

Introduction

Recently the genetic diversity of farm animals has more importance in the animal genetics. The new trend in genetic improvement programs is to investigate the differences among the breeds of the same species. All animal species, including poultry, produce free radicals as by-products of metabolism that could potentially damage or destroy biological molecules in cells. Superoxide dismutases, catalase, and glutathione peroxidases (GSHPx) are the most important antioxidant enzymes that destroy these free radicals before damage occurs (Özturk Ürek *et al.,* 2001). Cellular GSHPx is a key intracellular antioxidant enzyme that contains a selenocysteine residue at its active site (Handy *et al.,* 2006). The primary functions of the GSHPx iso-enzymes are to detoxify hydrogen peroxide and to convert lipid hydroperoxides to non-toxic alcohols (Jenkinson *et al.,* 1982). The activity of blood plasma GSHPx (BP-GSHPx) has been directly linked with dietary selenium level in most of the farm animals, including poultry (Payne and Southern, 2005). Antioxidants, such as GSHPx, play an important role in maintaining bird health, productivity and reproductive characteristics (Surai, 2002).

A genetic variation in GSHPx activity has been suspected previously in sheep (Langlands *et al.,* 1980; Atroshi and Sankari, 1981; Wiener *et al.,* 1983; Woolliams *et al.,* 1983), chicken (Cestnik, 1985; Cunningham *et al.,* 1987; Shen *et al.,* 1992; Shaaban *et al.,* 2003, 2004), goose (Mézes *et al.,* 1989), pig (Lingaas *et al.,* 1992), rabbit (Mézes *et al.,* 1994), and goat (Fidanci *et al.,* 2001). Several studies have suggested that most of the enzyme activities in animal tissues are affected by sex. Sex differ-

Received: November 12, 2007, Accepted: February 18, 2008
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ences in GSHPx activity may be the result of differences in distribution of selenium in male and female, or they may be caused by metabolic differences (Finley and Kincaid, 1991). There are some observations about the correlation of GSHPx activity and production traits, body weight (BW), weight gain, growth rate and wool production (Atroshi and Sankari, 1981; LaVorgna and Combs, 1983; Lingaas *et al.,* 1991; Mézes *et al.,* 1994). Also, there are some investigations concerning to the GSHPx activity as a possible selection criteria in rabbit breeding as slight negative phenotypic correlation was found between carcass traits and GSHPx activity of red blood cell haemolysate (RBC-GSPHx) in rabbits (Virág *et al.,* 1996). The poultry industry has a history of using breed crosses and, more recently, strain crosses, mainly to take advantage of heterosis (Fairfull, 1990). Langlands *et al.* (1980) reported that heterosis affect partly at the gene expression level of GSHPx activity in whole blood, the heterosis as a percentage of the mid-parent values ranged from −11.3 to 25.5. The existence of genetic variation in the concentration of GSHPx activity of different animals suggests that GSHPx activity is genetically regulated.

In present study, an attempt was made to assess the relative importance of these factors by comparing GSHPx activity in different pure breeds and cross breeds of chickens to obtain some information on the possible genetic background of GSHPx activities in RBC and BP in these breed groups, and its correlation with BW and sex at standardized conditions. The objectives of this research were to investigate the phenotypic variations in the GSHPx activities in RBC and BP in four breeds and their crosses of chickens at the age of sexual maturity.

Materials and Methods

A total of 240 blood samples (20 males (♂) and 20 females (♀) of each breed) was collected from six genetic groups of chickens, namely New Hampshire (NH) and Naked Neck Plymouth (NNP) and their cross (NH×NNP), White Plymouth Rock (WPR) and Naked Neck New Hampshire (NNNH), and their cross (WPR×NNNH). The blood samples were taken at the same time from males and females at the age of sexual maturity. All birds were clinically healthy and kept in the same environmental conditions and given the same diet. Blood samples were collected by venipuncture into tubes containing 200 μL EDTA (0.2 mol/L) as anticoagulant. Freshly collected blood samples were centrifuged (15 min, 2,500 rpm), plasma was removed and stored frozen (−20 ℃) until analysed. Erythrocytes was haemolysed with nine-fold of their volume of redistilled water and by freezing (−20℃, 24 hours) and thawing (37℃, 30 min).

GSHPx activity was measured using reduced glutathione and cumene-hydroperoxide as co-substrates (Matkovics *et al.,* 1988). The enzyme activity was expressed in units reflecting the oxidation of reduced glutathione in nmoles per minute at 25℃ and was related to the protein content. Protein content of BP and RBCs were determined using biuret method (Weichselbaum, 1946).

Data of body weight, GSHPx activity in BP and RBC were analysed using two-ways ANOVA, according to the following model: $Y_{ijk}=M+G_i+S_j+(GS)_{ij}+e_{ijk}$. where: M = the common mean, G_i = the effect of the i^{th} breed group; S_j = the effect of the j^{th} sex: $(GS)_{ij}$ = the effect of interaction between the i^{th} breed group and the j^{th} sex; e_{ijk} = random error term, using the GLM procedure of SPSS program (SPSS, 1999). Means were compared for main effects and their interaction by Duncan's multiple range test (Duncan, 1955), when significant F values were obtained ($P<0.05$). Heterosis was calculated as a percentage of the midparent values. Correlation analyses were performed by using the CORR procedure from SPSS program (SPSS, 1999) and Spearman correlation coefficient was used.

Results

Phenotypic Variations of Body Weight

Concerning breed effect, significant differences were found among the breeds showing the highest average BW in NNP (1.97 kg) and the lowest in NH breed (1.11 kg). NH×NNP cross had higher BW than their parent NH and lower BW than their parent NNP, also the WPR×NNNH cross had higher BW than their parent NNNH and lower BW than their parent WPR as shown in Table 1. Sex had also significant influence on BW, males having higher values than females (Table 1). Breed and sex interaction showed that males had significantly higher BW than females in all breed groups as shown in Table 2.

Phenotypic Variations of GSHPx Activity in Red Blood Cell Haemolysate

Regarding the breed effect, WPR had highest activity (6.24 U/g protein), and NH had the lowest (4.70 U/g protein). NH×NNP cross had lower activity (3.98 U/g protein) than their parents NH and NNP (4.70 and 4.93 U/g protein), also WPR×NNNH cross had lower activity (4.83 U/g protein) than their parents WPR and NNNH (6.24 and 5.38 U/g protein) as shown in Table 1. Sex had, also significant influence on GSHPx activity in RBC, females having higher enzyme activity than males (Table 1). Regarding breed and sex interaction, females had higher RBC-GSHPx activity than males in all breed groups except for NNP breed as shown in Table 2.

Phenotypic Variations of GSHPx Activity in Blood Plasma

Concerning breed effect, significant differences of GSHPx activity were found among the breeds showing the highest enzyme activity in WPR (7.08 U/g protein) and the lowest in NNNH breed (6.22 U/g protein). NH×NNP cross had lower insignificant activity (6.87 U/g protein) than their parents NH and NNP (7.00 and 6.90 U/g protein) while, the WPR×NNNH cross had significant higher activity (7.23 U/g protein) than their parents WPR and NNNH breeds (7.08 and 6.22 U/g protein) as shown in Table 1. Sex had also significant influence on BP-GSHPx activity, males having higher GSHPx activity

Table 1. **Body weight (BW), GSHPx activity in red blood cells (RBC) and blood plasma (BP) in different breed and sex groups of chickens (means±SEM)**

Breed	BW kg	RBCs GSHP×activity (U/g protein content)	BP
Breed effect			
NH	1.11±0.05[d]	4.70±0.33[c]	7.00±0.41[ab]
NNP	1.79±0.08[a]	4.93±0.24[b]	6.90±0.24[ab]
NHt×NNP	1.46±0.05[b]	3.98±0.23[c]	6.87±0.28[ab]
WPR	1.53±0.06[b]	6.24±0.54[a]	7.08±0.31[ab]
NNNH	1.26±0.05[c]	5.38±0.39[ab]	6.22±0.37[b]
WPR×NNNH	1.47±0.07[b]	4.83±0.27[b]	7.23±0.33[a]
Sex effect			
♂	1.61±0.04[a]	4.32±0.32[b]	7.35±0.27[a]
♀	1.22±0.03[b]	5.62±0.35[a]	6.43±0.30[b]

[a, b] Different letters in each column means significant difference ($P<0.05$).

Table 2. **Breed by sex interactions of body weight (BW), GSHPx activity in red blood cells (RBC) and blood plasma (BP) in chickens (means±SEM)**

Breed	Sex	BW (kg)	RBC GSHPx (U/g protein content)	BP
NH	♂	1.26±0.06[ef]	4.43±0.56[cd]	6.95±0.47[a]
	♀	0.95±0.05[g]	4.99±0.34[c]	7.04±0.71[a]
NNP	♂	1.99±0.07[a]	5.10±0.38[c]	7.22±0.11[a]
	♀	1.46±0.06[cd]	4.66±0.28[cd]	6.38±0.52[ab]
NH×NNP	♂	1.62±0.07[c]	3.60±0.36[d]	7.49±0.29[a]
	♀	1.33±0.04[de]	4.28±0.28[cd]	6.38±0.40[ab]
WPR	♂	1.78±0.03[b]	4.27±0.49[cd]	7.45±0.49[a]
	♀	1.31±0.04[de]	7.94±0.48[a]	6.76±0.34[ab]
NNNH	♂	1.41±0.06[de]	4.21±0.16[cd]	7.16±0.52[a]
	♀	1.10±0.03[fg]	6.66±0.55[b]	5.18±0.28[b]
WPR×NNNH	♂	1.79±0.04[b]	4.30±0.30[cd]	7.80±0.44[a]
	♀	1.25±0.07[ef]	5.20±0.41[c]	6.83±0.45[ab]

[a, b] Different letters in the same column means significant difference ($P<0.05$).

than females (Table 1). Breed and sex interaction showed that males had higher BP-GSHPx activity than females in all breed groups except for NH breed (Table 2).

Heterosis Effect

Means of heterosis estimates percentage (as a percentage of the midparent values) of NH×NNP and WPR×NNNH crosses for GSHPx activities in BP and RBC, and BW are presented in Table 3. Heterosis estimates for GSHPx activities in BP and RBC, and BW in males, females and both sexes indicated that males had higher percentages than females for GSHPx activities in BP and RBC in NH×NNP cross, while the opposite was found for WPR×NNNH cross as shown in Table 3. All heterosis percentages for RBC-GSHPx activity were negative and had high values except for males of WPR×NNNH cross. Heterosis estimates for BP-GSHPx activity were negative for NH×NNP cross, while the opposite was found for WPR×NNNH cross. All heterosis estimates for BW were positive and had high percentages except for males of NH×NNP cross (Table 3).

Phenotypic Correlation between GSHPx Activities in Red Blood Cells Haemolysate and Blood Plasma with Body Weight

Regarding the breed effect of GSHPx activity, negative correlations were found for all breeds except for NNP. Significant negative correlations were found between BW and RBC-GSHPx activity for WPR and NNNH (−0.68 and −0.67; $P<0.01$) as shown in Table 4. The overall of all observations showed significant negative correlation between BW and RBC-GSHPx activity (Table 4).

The phenotypic correlations between BW and BP-GSHPx activity of the various breeds had different values (Table 4). Positive correlations were found for all breeds except for NNP. The overall of all observations showed

Table 3. **Mean estimated heterosis effect computed as a percentage of the mid-parent values of NH×NNP and WPR×NNNH crosses of GSHPx activity in red blood cells (RBC) and blood plasma (BP), and body weight (BW)**

Breed	Sex	Heterosis%		
		RBC	BP	BW
NHt×NNP	♂	−24.45	−5.72	−0.38
	♀	−11.30	−4.92	+10.37
	(♂+♀)	−17.34	−1.15	+0.69
WPR×NNNH	♂	+1.42	+6.78	+12.23
	♀	−28.77	+14.41	+3.73
	(♂+♀)	−16.87	+8.73	+5.38

Table 4. **Phenotypic correlation between GSHPx activity in red blood cells (RBC) and blood plasma (BP), and body weight (BW)**

Breed	RBC	BP
NH	−0.35	−0.07
NNP	0.04	0.08
NH×NNP	−0.24	0.16
WPR	−0.68**	0.08
NNNH	−0.67**	0.40
WPR×NNNH	−0.27	0.08
Overall mean	−0.27**	0.12

** Correlation is significant at $P<0.01$ level.

that, positive correlation was found between BW and BP-GSHPx activity as shown in Table 4.

Phenotypic Correlation between GSHPx Activity in Blood Plasma and Red Blood Cell Haemolysate

Significant positive correlation between GSHPx activity in BP and RBC was found (0.35; $P<0.01$).

Discussion

Several studies pointed out that the expression of that antioxidant enzyme, GSHPx, varies according to genetic background, sex, age, physiological status, site of organs and diet (Lingaas *et al.,* 1991; Mote *et al.,* 1991; Nijhoff and Peters, 1992; Egaas *et al.,* 1995). Especially, genetic background and sex differences in antioxidant defence mechanism are of importance from many points of view such as cancer development, toxicology, and health of animals (Lingaas *et al.,* 1991; Van Lieshout and Peters, 1998; Jang *et al.,* 2001). The existence of genetic variation in the GSHPx activity in blood, liver and RBC of different animals suggests that GSHPx activity, beside other factors (Erdélyi *et al.,* 2001), is genetically regulated.

Environment and diet was the same for all breeds. This suggests that the observed differences among them are likely genetic ones. Thus, the results appear to support those of Langlands *et al.* (1980), Woolliams *et al.* (1983) and Fidanci *et al.* (2001), who reported breed differences in the GSHPx activity in the whole blood of sheep, cattle and goat. Also the present results are in agreement with those reported by Cestnik (1985) who found significant differences in the GSHPx in whole blood between two breeds of chickens (Rhode Island Red and Prelux-Bro) during embryonic development, day-old age and at the period of egg production. Langlands *et al.* (1980) reported that heterosis affects partly at the gene expression level of whole blood GSHPx activity, the heterosis as a percentage of the mid-parent values ranged from −11.3 to 25.5, which is in agreement of present results.

Few previous works has been done on the correlation between production traits and GSHPx activity. It is concluded that, the correlations depends on the species and tissue. Also, present results show that, the correlations are influenced by breeds and tissues.

Negative correlations between BW and RBC-GSHPx activity which were obtained in present experiment are similar to the results reported by Atroshi and Sankari (1981) in sheep, LaVronga and Combs (1982) in chicken, Lingaas *et al.* (1991) in pig, Mézes *et al.* (1994) and Virág *et al.* (1996) in rabbit. The significant negative correlation between BW and RBC-GSHPx activity may represent an adaptation mechanism to relatively low selenium intake particularly in animals with higher growth rate (Atroshi and Sankari, 1981) but further research is needed to prove that hypothesis. Also further studies are needed to investigate the other antioxidant enzymes and its correlation with production traits. Contrary to RBC, BP-GSHPx activity showed positive correlation with BW in most of the breeds investigated. That difference probably caused by the different origin of the enzyme molecule. RBC-GSHPx synthesise in the red blood cells while BP-GSHPx in the kidney tubular cells and it depends on the actual selenium supply less than the RBC-GSHPx, but the enzyme synthesis, and consequently the BP-GSHPx activity depends on the integrity of kidney tubular epithelial cells (Avissar *et al.,*1994). Phenotypic correlation between GSHPx activity in BP and RBC is not reported before,

present data indicated that, the measure of the enzyme activity in one tissue could be used as a good indicator to the GSHPx activity in other tissues based one the high correlation values which obtained in present results but further research need to prove this hypothesis.

In conclusion, phenotypic variation in the GSHPx activity may have given an opportunity for selection among breeds. Based on, heterosis percentages, cross-breeding plays a part in the expression of enzyme activity. Significant negative correlation between body weight and RBC-GSHPx activity may represent an adaptation to low selenium intake, so when selenium deficiency diseases such as nutritional muscular degeneration are common. Further research is needed to establish if the low enzyme activity really represent an adaptation to low selenium intake. It would be important to pinpoint the biochemical difference, if alternative non-selenium dependent pathways of peroxide detoxification occur in the low enzyme activity. Such information would be important in the search for the possibility of genetically selecting birds and animals resistant to selenium deficiency.

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