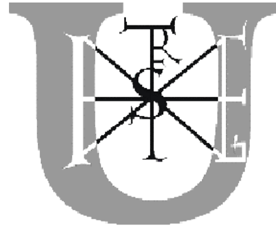


SZENT ISTVÁN UNIVERSITY
FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

PHENOTYPIC VARIATION OF GLUTATHIONE PEROXIDASE ACTIVITY IN DIFFERENT GENOTYPES OF CHICKEN AND ITS CORRELATION WITH SOME PRODUCTION TRAITS

Ph. D. Dissertation

Gihan Shaaban Farahat

Gödöllő
Hungary

2003

Ph. D. Program

Title:	**Animal Science**
Science:	**Agricultural Science**
Program Leader :	**Prof. László Horváth, DSc.** Department of Fish Culture, Szent István University, Faculty of Agricultural and Environmental Sciences, Gödöllő, Hungary
Supervisor:	**Dr. András Hidas, PhD.** Department of Poultry Breeding, Institute for Small Animal Research, Gödöllő, Hungary

Approval of Program Leader **Approval of Supervisor**

CONTENTS

1. INTRODUCTION......7

2. RESEARCH OBJECTIVES......10

3. LITERATURE REVIEW......11

3. 1. Free radicals and antioxidant defence......11
3. 1. 1. What are free radicals?......11
3. 1. 2. Biological role of free radicals......11
3. 1. 3. Production of free radicals......12
Abbreviations:......13
3. 1. 4. Scavenging of free radicals......15
Abbreviations:......16
3. 1. 5. Assessment of free radical activity......19

3. 2. Glutathione peroxidase in biological system......20
3. 2. 1. Molecular weight and subunits of GSHPx......21
3. 2. 2. Types of selenium-containing GSHPx......21
3. 2. 3. Non-selenium-dependent GSHPx......24
3. 2. 4. GSHPx-like protein......24
3. 2. 5. Hypothetical mechanism of action of glutathione peroxidase......24
3. 2. 6. Established function of GSHPx in living systems......27
3. 2. 7. Prevention of lipid peroxidation by glutathione peroxidase......28

3. 3. Genetics of GSHPx enzyme activity......30
3. 3. 1. Phenotypic variation of GSHPx enzyme activity in different animal species......30
3. 3. 2. Phenotypic variation of GSHPx enzyme activity within some animal species......32
Sheep:......33
Cattle:......34

3. 4. Effect of sex on GSHPx activity......36

3. 5. Effect of age on GSHPx activity......37

3. 6. Phenotypic correlation between GSHPx activity and production traits......40

3. 7. GSHPx activity and embryonic development......41

4. MATERIALS AND METHODS......43

4. 1. Animals......43

4. 2. Experimental conditions......43

4. 3. Experimental design......44
4. 3. 1. Experiment 1: GSHPx activity in chicken embryo......44
4. 3. 2. Experiment 2: Phenotypic variation of GSHPx activity in different breeds of different age groups, from 1-day till the age of highest egg production......44
4. 3. 3. Experiment 3: Phenotypic variation in the activity of GSHPx in two chicken breeds and their crosses......45

4. 4. Production traits......45

4. 5. Blood and liver samples......45

4. 6. Biochemical methods......46

4. 7. Statistical analysis......46

5. RESULTS......48

5. 1. Experiment 1: GSHPx activity in chicken embryo......48
5. 1. 1. Phenotypic variation of GSHPx activity of embryo liver......48
5. 1. 2. Phenotypic variation of GSHPx activity of RBC......53
5. 1. 3. Phenotypic variation of GSHPx activity of blood plasma......54
5. 1. 4. Phenotypic variation of egg weight......55
5. 1. 5. Phenotypic variation of body weight of day old chicken......57
NNP......57
5. 1. 7. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity......59

5. 2. Experiment 2: Phenotypic variation of GSHPx activity in different breeds of different age groups, from day-old to the age of highest egg production......60
5. 2. 1. Phenotypic variation of GSHPx activity of liver homogenate......60
5. 2. 3. Phenotypic variation of GSHPx activity of blood plasma......67
5. 2. 4. Phenotypic variation of body weight......71
5. 2. 5. Phenotypic correlation between body weight and liver, RBC and blood plasma GSHPx activity......72
5. 2. 6. Phenotypic correlation between egg production and liver, RBC and blood plasma GSHPx activity......72
5. 2. 7. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity......72

5. 3. Experiment 3: Phenotypic variation in the GSHPx activity in two chicken breeds and their crosses of different ages from 1-day to the age of sexual maturation......75
5. 3. 1. Phenotypic variation of liver GSHPx activity......75
5. 3. 4. Phenotypic variation of body weight 85
5. 3. 5. Heterosis and reciprocal effects......85
5. 3. 6. Phenotypic correlation between GSHPx activity in RBC haemolysate and blood plasma with body weight......87
5. 3. 7. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity......87

6. DISCUSSION......89

6. 1. Genetic differences in GSHPx activity......89

6. 2. Effect of sex on GSHPx activity......92

6. 3. Effect of age on GSHPx activity......92

6. 4. Phenotypic correlation between production traits and GSHPx activity......93

6. 5. Phenotypic correlations between liver, RBC and blood plasma GSHPx activity......94

6. 6. Suggestions for further research......94

7. SUMMARY......95

8. NEW SCIENTIFIC RESULTS......97

9. PUBLICATIONS RELATED TO THE SUBJECT OF DISSERTATION......98

CONFERENCE PRESENTATIONS:..99

10. REFERENCES...100

11. ACKNOWLEDGEMENTS...119

12. APPENDIX...120

..122

..122

ABBREVIATIONS

ADP	Adenosine diphosphate
BW	Body weight
EW	Egg weight
FAD	Flavin adenine dinucleotide
FAD	Flavin adenine dinucleotide (oxidized)
$FADH_2$	Flavin adenine dinucleotide (reduced)
FMN	Flavin Mononucleotide
GSH	Glutathione
GSHPx	Glutathione peroxidase
GS-SG	Oxidized glutathione (glutathion disulfide)
H_2O_2	Hydrogen peroxide
HEP	Age at highest egg production of layers
HOCl	Hydrochlorous acid
HS	Hungarian Speckled breed
HW	Hungarian White breed
L•	Lipid radical
LO•	Lipid alkoxyl radical
LOO•	Lipid peroxyl radical
LOOH	lipid hydroperoxide
N x T	New Hampshire sires crossed Transylvanian Naked Neck Black dames breed
NH	New Hampshire breed
NNNH	Naked Neck New Hampshire breed
NNP	Naked Neck Plymouth breed
NO	Nitrogen oxide
O^{-}_2	Superoxide anion radical
•OH	Hydroxyl radical
$ONOO^{-}$	Peroxynitrite
PRW	Plymouth Rock White breed
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell haemolysate
RNS	Reactive nitrogen species
ROO•	Peroxyl radicals
ROS	Reactive oxygen species
Se	Selenium
Se-GSHPx	Selenium-dependent glutathion peroxidase
SM	Age at sexual maturation
SOD	Superoxide dismutase
T x N	Transylvanian Naked Neck Black sires crossed New Hampshire dames breed
TNNB	Transylvanian Naked Neck Black breed
TNNW	Transylvanian Naked Neck White breed
Vit C	Vitamin C (ascorbic acid)
Vit E	Vitamin E (α-tocopherol)

1. INTRODUCTION

Several different reactions are required for the maintenance of normal metabolism and the production of energy in the cell, which also produce potentially toxic free radicals as unwanted by-products *(Chester and Arthur, 1988)*. Normally, the body is protected against reactive oxygen metabolites and their toxic products by a wide range of known defense mechanisms, like antioxidant enzymes.

Antioxidants play an important role in maintaining the health, productivity and reproductive characteristics of the animals. In general, an integrated antioxidant system has been described in avian tissues *(Surai, 1999a; 2002)*; and it has been suggested that the cell's first line of cellular antioxidant defense is based on the activity of three enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase. In this respect GSHPx has received only limited attention in relation to poultry production. However, during recent years the importance of this enzyme in the antioxidant protection of tissues has become increasingly appreciated.

Glutathione peroxidases are substantially more efficient on a molar basis than other enzymes *(Michiels et al., 1994)*. At least five forms of glutathione peroxidases have been reported; therefore it is called more like an enzyme family than a single enzyme. They are present in almost every cell of animals; they are different in many properties, including their localization, subunit structure, primary structure and enzymatic nature *(Arthur, 2000)*.

Chick embryo tissues are characterised by high concentration of polyunsaturated fatty acids *(Noble and Cocchi, 1990)* and are very sensitive to lipid peroxidation *(Gaál et al., 1995)*, therefore defence from lipid peroxidation is a crucial task for the embryo. The antioxidant system of the chick embryo consists of a combination of natural metabolites *(Gaál et al., 1995)* and antioxidant enzymes *(Wilson et al., 1992)*. Antioxidant enzymes are the major cell defence against acute oxygen toxicity *(Harris, 1992)*, the expression of their activity is regulated in tissue-specific fashion at the gene level *(Bermano et al., 1995; Tsan, 1997; Weiss et al., 1997)*. Information concerning antioxidant enzymes in the chick embryos is limited *(Surai, 2002)*.

A genetic variation in GSHPx activity has been suspected previously in pig *(Lingaas et al., 1991; 1992)*, chicken *(Cestnik, 1985; Cunningham et al., 1987; Shen et al., 1992)*, goose *(Mézes et al., 1989)*, rabbit *(Mézes et al., 1994a)*, sheep *(Langlands et al., 1980; Atroshi and Sankari, 1981; Woolliams et al., 1983)* and goat *(Fidanci et al., 2001)*. *Tucker and Kilgour (1970)* have shown that reduced glutathione (co-substrate of GSHPx)

concentration is controlled by a single pair of alleles and *Andrewartha (1978)* has reported a positive relation between glutathione concentration and GSHPx activity. Such an relation might explain the origin of the breed differences. Genetic influences have previously been described as important factors in selenium deficiency in chicken; resulting in some strains or individuals being more susceptible to selenium deficiency than others *(LaVronga and Combs, 1982)*.

There are some observations about the correlation of GSHPx activity and production traits, body weight, weight gain, growth rate and wool production. *(Atroshi and Sankari, 1981; LaVronga and Combs, 1982; Lingaas et al., 1991; Mézes et al., 1994a)*. 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 enzyme activity of erythrocytes in rabbits *(Virág et al., 1996)*.

Several studies have suggested that many enzymes activity in animal tissues are affected by sex and age. Sex differences may be the result of differences in distribution of selenium in male and female, or by the well-known metabolic differences between the two sexes *(Finley and Kincaid, 1991)*. According to the free radical ageing theory, ageing can be considered as a process of irreversible changes associated with an accumulation or integration of free radicals induced by damages in the cell *(Harman, 1956)*.

In some farm animal species, evidence of unfavourable genetic correlation between production traits and disease resistance has been found *(Syväjärvi et al., 1986; Edfors-Lilja et al., 1986)*. It is therefore important to be aware of genetic changes which are correlated to selection for improved performance. Hence, screening of parameters prove to have genetic variation, especially those correlated to performance and disease resistance, like in this study, may indicate the importance of indirect selection.

The value of making comparative studies is that they allow searching for common patterns of enzyme distribution. Any general pattern of glutathione peroxidase distribution would presumably promote an understanding of its biological function, especially if this distribution could be correlated with other enzymes and/or metabolic cofactors. Another value of making comparative studies is that animal and tissue sources with high GSHPx activity can be identified.

The existence of genetic variation in the concentration of GSHPx activity in blood, liver and RBC of different animals suggests that GSHPx activity is genetically regulated. In my study presented in the thesis an attempt was made to assess the relative importance of these factors by comparing GSHPx activity of different chicken breeds and crosses to

obtain some information on the possible genetic background of liver homogenate, erythrocyte haemolysate and blood plasma GSHPx enzyme activity in different genotypes of chicken, and its correlation with some production traits, age and sex at standardised condition, which has not been reported before in detail in chicken.

2. RESEARCH OBJECTIVES

The objectives of the investigation were:

1. Measurement of the phenotypic variation of liver GSHPx enzyme activity in chicken embryo's in different genotypes during the embrionic development at standardised conditions.

2. Measurement of the phenotypic variation of GSHPx enzyme activity of liver, red blood cells and blood plasma in different chicken genotypes and their crosses from day-old age up to the age of highest egg production at standardised conditions.

3. Measurement of the effect of heterosis and reciprocal effect on the GSHPx enzyme activity of liver, red blood cells and blood plasma and on body weight.

4. Measurement of the effect of age and sex on GSHPx enzyme activity of liver, red blood cells and blood plasma during embrionic development and from day-old age up to the age of highest egg production at standardised conditions.

5. Estimate of the correlation among different production traits and GSHPx enzyme activity of liver, red blood cells and blood plasma.

3. LITERATURE REVIEW

3. 1. Free radicals and antioxidant defence

3. 1. 1. What are free radicals?

Free radicals are defined as molecules having an unpaired electron in the outer orbital *(Gilbert, 2000)*. They are generally unstable and very reactive. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl ($RO•_2$), alkoxyl (RO•), and hydroperoxyl ($HO•_2$) radicals. Nitric oxide and nitrogen dioxide ($•NO_2$) are two nitrogen free radicals. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), hypobromous acid (HOBr), and peroxynitrite ($ONOO^-$). Reactive oxygen, reactive nitrogen species and reactive chlorine species are produced in animals and humans under physiological and pathological conditions *(Evans and Halliwell, 2001)*.

3. 1. 2. Biological role of free radicals

Free radicals play an important role in the origin of life and biological evolution, implicating their beneficial effects on the organisms *(McCord, 2000)*. For example, oxygen radicals exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate-cyclase activity in cells *(Lander, 1997; Zheng and Storz, 2000)*. Also, nitrogen oxide (NO) is one of the most widespread signaling molecules and participates in virtually every cellular and organ function in the body *(Ignarro et al., 1999)*. Physiological level of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, vascular tone, and hemodynamics *(Ignarro et al., 1999)*. In addition, NO produced by neurons serves as a neurotransmitter, and NO generated by activated macrophages is an important mediator of the immune response *(Fridovich, 1999)*. However, as oxidants and inhibitors of enzymes containing an iron-sulfur center, free radicals and other reactive species cause the oxidation of biomolecules (e. g., protein, amino acids, lipid and DNA), which leads to cell injury and death *(Fridovich, 1999; McCord, 2000)*. For example, radiation-induced ROS markedly alter the physical, chemical, and immunological properties of SOD *(Fang, 1991)*, which further exacerbates oxidative damage in cells. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but is responsible for killing

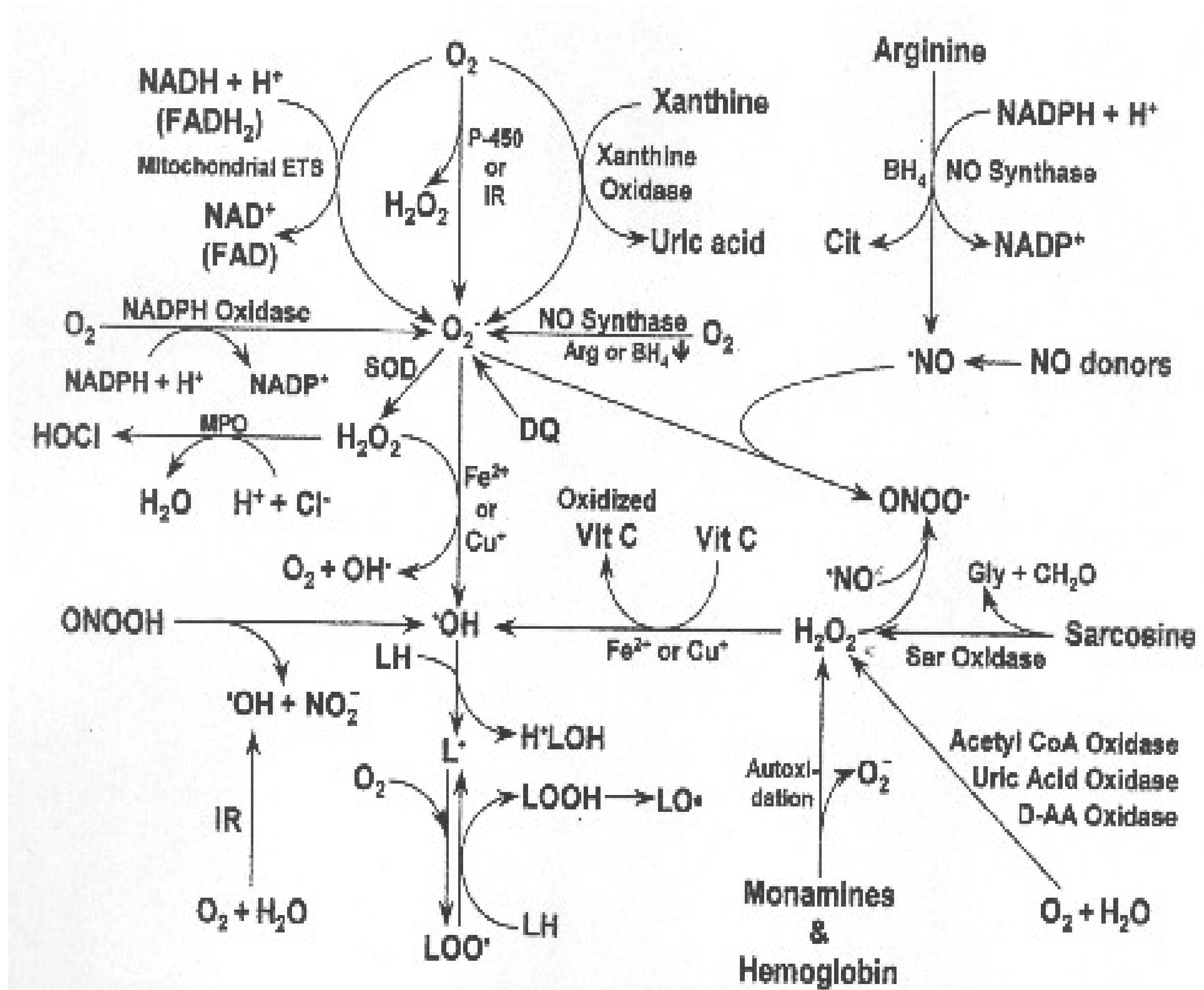
pathogens by activated macrophages and other phagocytes in the immune system *(McCord, 2000)*. Thus, there are two faces of free radicals in biology in that they serve as signaling and regulatory molecules at physiologic levels, but as highly deleterious and cytotoxic oxidants at pathologic levels *(Fridovich, 1999)*.

3. 1. 3. Production of free radicals

Oxygen is required for the generation of all reactive oxygen and nitrogen also reactive chlorine species *(Fridovich, 1999)*. The major reaction for the production of oxygen and nitrogen free radicals in the body is illustrated in Fig. 1. Nitrogen oxide is formed from L-arginine by one of the three NO synthase (NOS) isoforms: nNOS (originally identified as constitutive in neuronal tissue; also known as NOS-I or NOS-1), iNOS (originally identified as being inducible by cytokines in activated macrophages and liver, also known as NOS-II or NOS-2), and eNOS (originally identified as constitutive in vascular endothelial cells, also known as NOS-III or NOS-3) *(Wu and Morris, 1998)*. All NOS isoforms require oxygen, tetrahydrobiopterin, nicotinamide adenine dinucleotide phosphate (NADPH), calmodulin, flavin adenine dinucleotide (oxidized, FAD), flavin mononucleotide (FMN), and heme for catalytic activity, where as Ca^{2+} is essential for nNOS and eNOS activity. In contrast, superoxide is generated from O_2 by multiple pathways:

- NADPH oxidation by NADPH oxidase,
- Oxidation of xanthine or hypoxanthine by xanthine oxidase,
- Oxidation of reducing equivalents (e. g. nicotinamide adenine dinucleotide [reduced, NADH], NADPH, and $FADH_2$ [FAD reduced]) *via* the mitochondrial electron transport system,
- Autoxidation of monamines (e. g., dopamine, epinephrine, and norepinephrine), flavins, and hemoglobin in the presence of trace amounts of transition metals,
- One-electron reduction of O_2 by cytochrome P-450,
- One-electron reduction of O_2 by nNOS or eNOS when arginine or tetrahydrobiopterin is deficient *(Evans and Halliwell, 2001; Fridovich, 1999; Gilbert, 2000; Wu and Morris, 1998)*.

Fig. 1. Production of oxygen and nitrogen free radicals and other reactive species ***(Adapted from Fang, 2002)***



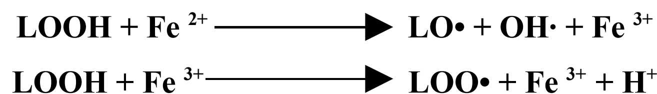
Abbreviations:

AA, amino acid; Arg, L-arginine; BH_4, (6R)-5,6,7,8,-tetrahydro-L-biopterin; CH_2O, formaldehyde; Cit, L-citrulline; DQ, diquat; ETS, electron transport system; FAD, flavin adenine dinucleotide (oxidized); $FADH_2$, flavin adenine dinucleotide (reduced); Gly, glycine; H_2O_2, hydrogen peroxide; HOCl, hydrochlorous acid; H•LOH, hydroxy lipid radical; IR, ionizing radiation; L• lipid radical; LH, lipid (unsaturated fatty acid); LO•, lipid alkoxyl radical; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide; MPO, myeloperoxidase; NAD^+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidized); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); •NO, nitric oxide; O^-_2 superoxide anion radical; •OH hydroxyl radical; $ONOO^-$, peroxynitrite; P-450, cytochrome P-450; PDG, phosphate-dependent glutaminase; Sar, Sarcosine; SOD, superoxide dismutase; Vit C, vitamin C (ascorbic acid); Vit E, vitamin E (α-tocopherol).

Superoxidase dismutase converts O_2^- to H_2O_2. Hydrogen peroxide is also produced through two-electron reduction of O_2 by cytochrome P-450, D-amino acid oxidase, acetyl coenzyme A oxidase, or uric acid oxidase *(Evans and Halliwell, 2001; Fang, 2002; Fridovich, 1999)*. Further, the oxidation of sarcosine in the pathway of glycine metabolism leads to H_2O_2 formation (Fig. 1).

In the presence of water and oxygen, ionizing radiation results in the production of O_2^-, H_2O_2, and •OH, with •OH being the major deleterious ROS. NO can react with O_2^- or H_2O_2 to form $ONOO^-$, whose oxidant potential is greater than that of O_2^- or H_2O_2 alone *(Fridovich, 1999; McCord 2000)*. As a strong oxidant, HOCl is generated from H_2O_2 and Cl^- by myeloperoxidase (a heme enzyme) particularly in immunologically activated phagocytes.

When free radicals and other reactive species (e. g. •OH, HOO•, $ONOO^-$) extract a hydrogen atom from an unsaturated fatty acid chain (e. g. n-6 polyunsaturated fatty acids), a carbon-centered lipid radical (L•) is produced. This is followed by the addition of oxygen to L• to yield a lipid peroxyl radical (LOO•). LOO• further propagates the peroxidation chain reaction by abstracting a hydrogen atom from a nearby unsaturated fatty acid. The resulting lipid hydroperoxide (LOOH) can easily decompose to form a lipid alkoxyl radical (LO•). This series of ROS-initiated lipid peroxidation reactions with the production of lipid peroxyl and alkoxyl radicals, collectively called chain propagation, occurs in cells, such that oxygen free radicals cause damage far in excess of their initial reaction products. Also lipid hydroperoxides are unstable and in the presence of transition metal ions can decompose to produce new free radicals and cytotoxic aldehydes:



$$\mathbf{LOOH + Fe^{2+} \longrightarrow LO\bullet + OH\cdot + Fe^{3+}}$$

$$\mathbf{LOOH + Fe^{3+} \longrightarrow LOO\bullet + Fe^{3+} + H^{+}}$$

These reactions account for much of the stimulating of lipid peroxidation by transition metal ions in biological systems *(Halliwell and Gutteridge, 1999)*.

The mitochondrial electron transport system is a source of superoxide anion *(Fridovich, 1999)*. Because NADH, NADPH, and $FADH_2$ are produced almost exclusively via the aerobic metabolism of protein, fat, and glucose, an increase in dietary energy intake enhances mitochondrial free radical production, which results in oxidative stress (Fig. 1). Thus calorie restriction reduces the generation of free radical species and retards aging in animals *(Sohal and Weindruch, 1996)*.

Throughout the life cycle, any animal may be at a risk of oxidative stress induced by high rates of oxygen use, the autoimmune activation of the immune system cells and environmental factors.

Free radical formation is considered to be a patho-biochemical mechanism involved in the initiation or progression phase of various human diseases including cardiovascular disease, some forms of cancer, cataracts, age-related mascular degeneration, rheumatoid arthritis and a variety of neurodegenerative diseases *(Hogg, 1998; Knight, 1998; Morrissey and O'Brien, 1998)*. In animal production free radical generation and lipid peroxidation are responsible for the development of various diseases as well as for a decrease in animal productivity and product quality *(Bottje and Wideman, 1995; Hurley and Doane, 1989; McDowell, 2000; Surai and Dvorska, 2001; Weiss, 1998)*.

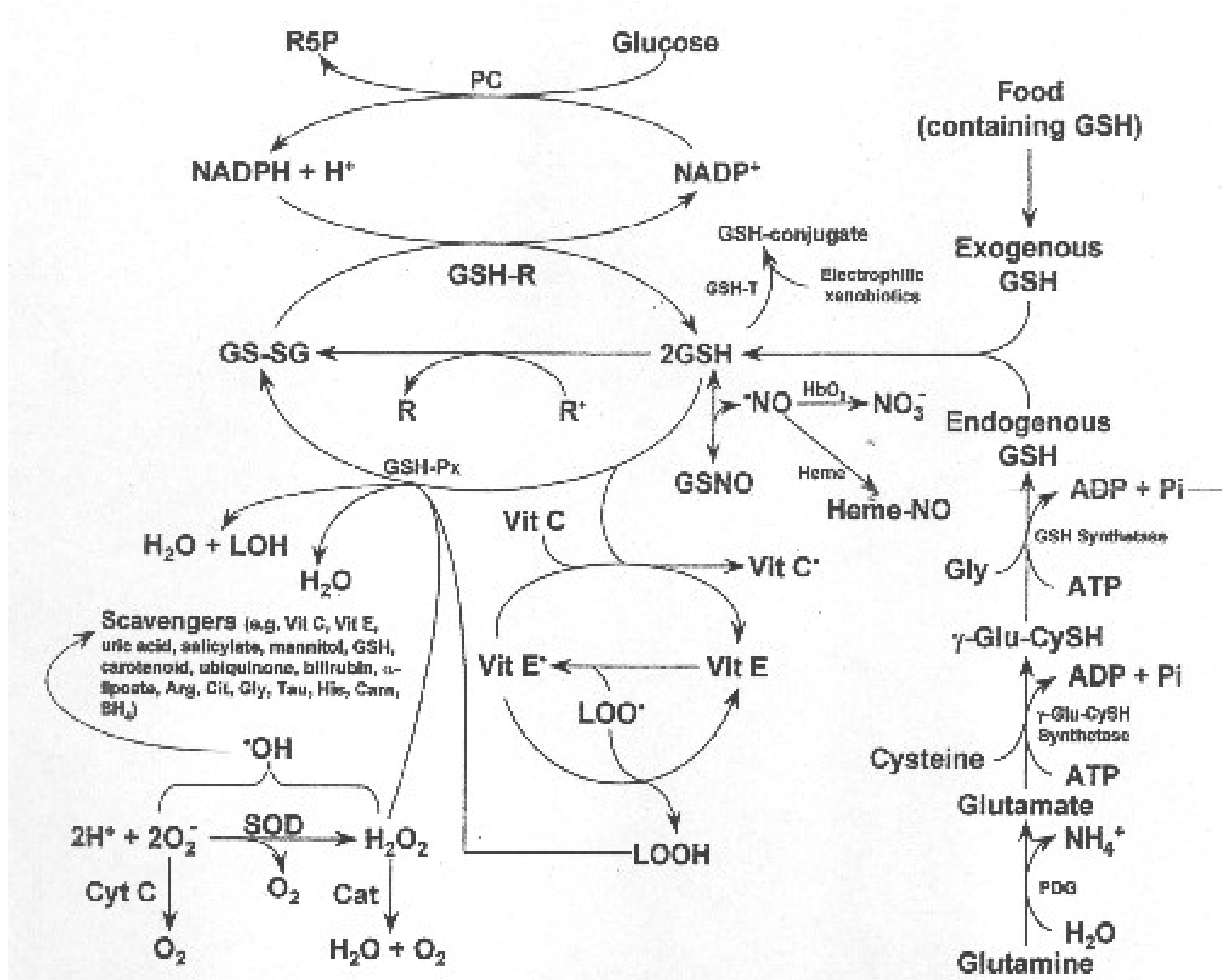
Prolonged exposure to free radicals, even at a low concentration may result in the damage of biologically important molecules and potentially leads to DNA mutation, tissue injury, and diseases *(Fridovich, 1999; McCord, 2000)*. Thus, although molecular oxygen is absolutely essential for aerobic life, it can be toxic under certain conditions. This phenomenon has been termed the *oxygen paradox*.

3. 1. 4. Scavenging of free radicals

The removal of free radicals is achieved through enzymatic and non-enzymatic reactions (Fig. 2).

NO is rapidly oxidized by oxyhemoglobin to form NO_3 (nitrate), the major stable oxidation end and product of NO in the body *(Wu et al., 1999)*. NO also reacts with glutathione (reduced; GSH) to form nitrosothiol or with heme to yield heme-NO. Physiologically, nitrosothiol can serve as a vehicle to transport NO in plasma, thereby increasing the biological half-life of physiological concentrations of NO *(Rassaf et al., 2002)*. In addition, tyrosine residues of proteins can be nitrosylated by NO or its derivative peroxynitrite. Moreover, GSH can scavenge $ONOO^-$ with the formation of oxidized glutathione (GS-SG), which is converted back to GSH by the NADPH-dependent glutathione reductase *(Sies, 1999)*.

Fig. 2. Removal of oxygen and nitrogen free radicals and other reactive species ***(Adapted from Fang, 2002)***



Abbreviations:

AA, amino acid; Arg, L-arginine; BH_4, (6R)-5,6,7,8,-tetrahydro-L-biopterin; CH_2O, formaldehyde; Cit, L-citrulline; DQ, diquat; ETS, electron transport system; FAD, flavin adenine dinucleotide (oxidized); $FADH_2$, flavin adenine dinucleotide (reduced); Gly, glycine; H_2O_2, hydrogen peroxide; HOCl, hydrochlorous acid; H•LOH, hydroxy lipid radical; IR, ionizing radiation; L• lipid radical; LH, lipid (unsaturated fatty acid); LO•, lipid alkoxyl radical; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide; MPO, myeloperoxidase; NAD^+, nicotinamide adenine dinucleotide (oxidized); NADH, nicotinamide adenine dinucleotide (reduced); $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidized); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); •NO, nitric oxide; O^-_2 superoxide anion radical; •OH hydroxyl radical; $ONOO^-$, peroxynitrite; P-450, cytochrome P-450; PDG, phosphate-dependent glutaminase; Sar, Sarcosine; SOD, superoxide dismutase; Vit C, vitamin C (ascorbic acid); Vit E, vitamin E (α-tocopherol).

The antioxidant system in the cell is based on the three major levels of defence. SOD, GSHPx, catalase and metal-binding proteins from the first level of defence through prevention of free radical formation. Chain-breaking antioxidants (vitamin A, E, C, carotenoids, glutathione, uric acid etc.) belong to the second level of defence and deal with prevention and restriction of chain formation and propagation. A third level of antioxidant defence deals with damaged molecules in the cell as a result of free radical action and toxic products of their metabolism and includes various enzymatic systems responsible for repair or removal of the damaged molecules.

Enzymatic protection against ROS and the breakdown products of peroxidised lipids and oxidized DNA is provided by many enzymes such as SOD, GSHPx, glutathione reductase, catalase.

Antioxidant nutrients included: vitamin E which can transfer its phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA, thereby breaking the radical chain reaction and preventing the peroxidation of PUFA in cellular and subcellular membrane phospholipids.

Vitamin E is a major component of the antioxidant system and has received substantial attention in recent literature. However, less attention has been paid to the chemical reactions in which vitamin E exerts its antioxidant properties. It is well-known that vitamin E (Toc-OH) effectively scavenges peroxyl radicals (ROO•) in the following reaction:

ROO• + Toc-OH ⟶ ROOH + Toc-O•

As a result of this reaction a tocopheroxyl radical (Toc-O•) and a hydroperoxide (ROOH) are produced. The tocopheroxyl radical can be returned to the active form of tocopherol by recycling reactions with other antioxidants including ascorbic acid, glutathione, carotenoids and ubiquinol *(Surai, 1999b)*. The second product of antioxidant action of vitamin E is a hydroperoxide. Hydroperoxides are toxic substances and if not removed impair membrane structure and function *(Gutteridge and Halliwell, 1990)*. Therefore hydroperoxides must be removed from the cell in the same way as H_2O_2, but catalase cannot react with these compounds. Only Se-dependent GSHPx can convert these compounds into non-reactive products *(Flohé, 1999)*:

ROOH + 2GSH $\xrightarrow{\text{Se-GSHPx}}$ ROH + GSSG + ROH

Therefore it appears that as the major antioxidant in the biological system, vitamin E performs only half the job of removing free radicals and producing hydroperoxides. The second part of the process is dependent on the activity of GSHPx.

As a reducing agent, vitamin C reacts with a vitamin E radical to yield a vitamin C radical, while regenerating vitamin E. Like a vitamin E radical, a vitamin C radical is not a reactive species, because its unpaired electron is energetically stable *(Fang, 2002)*. A vitamin C radical is converted back to vitamin C by GSH.

Glutathione, the most abundant thiol-containing substance of low molecular weight in cells, is synthesized from glutamate, cysteine, and glycine. *N*-acetylcysteine is a stable, effective precursor of cysteine for intracellular GSH synthesis *(Sies, 1999)*. As a major component of the cellular antioxidant system, GSH has the following characteristics:

1. GSH in the diet can be partly absorbed from the small intestine and can be synthesized *de novo*, so that GSH is an exogenous and endogenous antioxidant.
2. Although glutathione radical (GS•) formed from the oxidation of GSH is a pro-oxidant radical, GS• can react with another GS• to yield GS-SG, which is then reduced to GSH by the NADPH-dependent glutathione reductase.
3. GSH can react with a variety of xenobiotic electrophylic compounds in the catalytic reaction of glutathione-S- transferase.
4. GSH effectively scavenges ROS (e. g. lipid peroxyl radical, peroxynitrite and H_2O_2) directly and indirectly through enzymatic reactions.
5. GSH can conjugate with NO, resulting in the formation of a S-nitroso-glutathione adduct, which is cleaved by the thioredoxin system to release GSH and NO.
6. GSH interacts with glutaredoxin and thioredoxin (thio-proteins), which play important roles in the regulation of cellular redox homeostasis.

Cytochrome C and SOD catalyze the formation of O_2 from O_2^{-} (Fig. 2). A co-product of SOD is H_2O_2, which is converted to H_2O by catalase and the GSHPx. Glutathione peroxidase detoxifies lipid hydroperoxides to alcohols. Another type of GSHPx (phospholipid peroxide GSHPx-4) acts on phospholipid peroxides in membrane structures *(Sies, 1999)*.

Other ROS and RNS scavengers include uric acid (a metabolite of purines), salicylate, mannitol, carotenoide, ubiquinone, bilirubin (a product of hemoglobin catabolism), α-lipoate, arginine, citrulline, glycine, taurine, histidine, creatine (a metabolite of arginine, glycine and methionine), carnosine (β-alanyl-L-histidine, which is abundant in skeletal muscle), tetrahydrobiopterin (a metabolite of guanosine triphosphate and thus

glutamine), phytate, and tea polyphenols *(Akashi et al., 2002; Fridovich, 1999; Lass, 2002; Lawler et al., 2002; Machilin and Bandito,1987; Redmond et al., 1996; Wu and Meininger, 2000)*.

Glucose metabolism via the pentose cycle plays a crucial role in providing NADPH and, hence, maintaining the normal ratio of GSH to GSSG and a normal redox state in cells. When the intracellular concentration of GSH decreases and that of GSSG increases, the cellular demand for NADPH increases markedly *(Sies, 1999)*. This necessiates an increase in glucose metabolism *via* the pentose cycle. A deficiency of intracellular NADPH may exacerbate an imbalance between the production and scavenging of free radicals. When rates of free radical production are greater than the scavenging rates, oxidative damage likely occurs in cells and tissues.

3. 1. 5. Assessment of free radical activity

There are multiple, complex methods for assessing free radical activity depending on experimental conditions, the availability of analytical facilities, and the investigator's interest *(Jackson, 1999)*. In view of the lack of any standard assays of free radical activity, three major approaches have been used for this purpose:

1. Determination of endogenous antioxidant levels

Most studies have examined the concentrations of antioxidants (e. g. vitamin E and C, carotenoids, folate, glutathione) in blood plasma and cells and the cellular activity of antioxidant enzymes (e. g. glutathione reductase, SOD, catalase and GSHPx). Because GSH is rapidly oxidized to GSSG by radicals and other reactive species and GSSG is exported from cells, intracellular [GSSG] / 2[GSH] ratio can provide a valid index of oxidative stress.

II. Measurement of the products of oxidized macromolecules.

Assessments of lipid peroxidation have included the analysis of lipid peroxides, isoprostanes, diene conjugates and breakdown products of lipids (e. g., malondialdehyde, ethane, pentane and 4-hydroxynonenal). Among these products malondialdehyde is often used as a reliable marker of lipid peroxidation. For assessing ROS-induced protein oxidation, most investigators have determined the production of protein carbonyls, the loss of free thiol groups in proteins, and nitration of protein-bound tyrosine residues *(Fang, 2002)*. Protein nitrotyrosine has been widely used as a convenient stable marker for the production of reactive nitrogen-centered (e. g., NO and peroxynitrite) oxidants *(Gilbert,*

2000). Specific products of DNA base oxidation such as 8-OH-deoxyguanosine, 5-OH cytosine; 8-OH adenine, 8-OH guanine and thymine glycol have been measured often to assess DNA base oxidation *(Jackson, 1999)*. Importantly, urinary excretion of 8-hydroxydeoxyguanosine may provide a useful, non-invasive means to assess whole-body DNA base oxidation in humans and animals *(Gilbert, 2000)*.

III. Direct detection of free radicals

Direct detection of free radicals has been performed using electron spin resonance and spin trapping techniques *(Fang, 1991)*. Although the electron spin resonance technique is suitable for detecting free radicals in chemistry, it has limited application to biological tissues owing to their usually high content of water. However, this problem can be overcome by the use of the spin trapping technique, which involves the conversion of highly reactive free radicals to relatively inert radicals, followed by electron spin resonance analysis *(Fang, 1991)*.

3. 2. Glutathione peroxidase in biological system

In general, an integrated antioxidant system has been described in avian tissues *(Surai, 1999a, 2002);* and it has been suggested that the first line of cellular antioxidant defence is based on the activity of three enzymes: SOD, glutathione peroxidase and catalase. In this respect GSHPx has received only limited attention in relation to poultry production. However, during recent years the importance of this enzyme in the antioxidant protection of tissues has become increasingly appreciated. Since the major form of GSHPx is selenium-dependent, the role of selenium in animal nutrition has attracted considerable attention *(Mahan, 1999)*.

Selenium is recognized as having anti-carcinogenic and antiviral properties and is known to have important roles in reproductive function and development, immunocompetence and aging. These selenium functions have been described recently in a series of comprehensive reviews. The role and responses to dietary selenium in poultry nutrition appeared during the *1970s;* and recent understanding of antioxidant system functions and new discoveries regarding the GSHPx enzyme family are the basis for further development in the selenium nutrition of poultry.

The importance of selenium in animal nutrition lies in the fact that both first (detoxification of H_2O_2 formed by SOD action) and second (detoxification of hydroperoxides) levels of antioxidant defence in the cell rely on the activity of Se-dependent GSHPx, which in turn depends on adequate Se status in the cell. Furthermore,

even at very high levels of dietary vitamin E there is a need for selenium *(Surai, 2000)*. This is in agreement with data showing that high levels of dietary vitamin E do not replace cellular GSHPx in protecting mice from acute oxidative stress *(Cheng et al., 1999)*. During selenium deficiency lipid peroxidation is accelerated and damage to biological molecules can be lethal for the cell *(Halliwell and Gutteridge, 1999)*.

A delicate antioxidant/prooxidant balance in the body is an important determinant of chicken health, embryonic development, sperm quality and probably productive and reproductive characteristics of poultry. There are different ways in which the antioxidant system can be altered or regulated. The most important regulation is the animal response to stress condition by synthesizing antioxidant enzymes, (e. g., SOD and GSHPx). However, this response will be effective only if cofactors such as selenium for GSHPx and Cu, Zn and Mn for SOD are available. Therefore, dietary Se is a crucial factor regulating GSHPx activity and the efficiency of the antioxidant system *(Surai,1999a; 2000; 2002; Surai and Dvorska, 2001)*.

3. 2. 1. Molecular weight and subunits of GSHPx

Classic glutathione peroxidase has a molecular weight of about 85000, consists of four apparently-identical subunits and contains four gram atom selenium/mol. The enzyme-bound selenium can undergo a substrate-induced redox change and is obviously essential for activity. In accordance with the assumption that a selenol group is reversibly oxidised during catalysis, ping-pong kinetics is observed *(Flohé, 1978)*.

3. 2. 2. Types of selenium-containing GSHPx

There are four known GSHPx isoforms which contain selenocysteine at the active site. Additionally, there are at least two other proteins with over 40% sequence identity to cytosolic GSHPx, but which do not contain selenocysteine. In addition to the selenium-containing GSHPx and the proteins which contain cysteine and have similar structures to the GSHPx, there are many molecules having selenium-independent GSHPx activity in mammalian systems. These activities are mainly associated with glutathione-S-transferases a family of enzymes with the activity directed mainly towards organic hydroperoxides and very little activity against hydrogen peroxide *(Hayes and Strange, 1995; Hayes and McLellan, 1999)*:

Mills first described GSHPx activity in *1957,* and its function was hypothesised to be protection of red blood cells against haemolysis by oxidation. This enzyme, originally

called glutathione peroxidase, has been called classical GSHPx and is now generally called GSHPx-1 [EC1.11.1.9.], in some publications this enzyme has also been called cytosolic or cellular GSHPx. GSHPx-1 can metabolise hydrogen peroxide and a range of organic peroxides, including cholesterol and long-chain fatty acid peroxides *(Sunde, 1988)*. However, unless accompanied by phospholipase activity to release the fatty acids, GSHPx-1 cannot metabolise fatty acid hydroperoxides in phospholipids *(Grossmann and Wendel, 1983)*. GSHPx-1 can interact with a wide range of organic hydroperoxides as well as hydrogen peroxide. Despite this range of peroxide substrates, it is very specific for glutathione as reducing substrate. Thus GSHPx-1 activity is often discussed in parallel with glutathione reductase activity, which maintains a constant supply of glutathione from GSSG for enzyme activity. GSHPx-1 has been sequenced either directly or through complementary DNA cloning in a range of species including human, mouse, rabbit, cattle and sheep *(Sunde, 1990)*. In all species examined GSHPx-1 is a tetrameric protein with four identical subunits, each of which contains one selenocysteine residue in a total molecular weight of 22-23 kDa. The three-dimensional structure of bovine GSHPx-1 shows that it contains four spherical subunits, each with a selenocysteine residue in a depression on the surface *(Epp et al., 1983)*. The proximity of each selenocysteine to an adjacent subunit suggests that the active site of the enzyme relies on all subunits. Initially, chemical derivatization was used to show that selenocysteine is at the active site of the enzyme *(Arthur, 2000)*. In common with other selenium-containing proteins, replacement of selenocysteine with cysteine at the active site of glutathione peroxidase causes a large decrease in enzyme activity *(Rocher et al., 1992)*. This is consistent with selenocysteine being a much more efficient redox catalyst than cysteine at physiological pH *(Arthur, 2000)*.

The second form of GSHPx which occurs in the cytosol and is tetrameric has been called gastrointestinal or GSHPxG1. This protein is now called GSHPx-2 and has approximately 65% amino acid sequence identity and 60% nucleotide sequence identity with GSHPx-1 *(Chu et al., 1993)*. Both GSHPx-1 and GSHPx-2 have similar substrate specificity in that they reduce hydrogen peroxide or fatty acid hydroperoxides rapidly but not phospholipid hydroperoxides. In the rat, GSHPx-2 messenger RNA is found mainly in the gastrointestinal tract, however, in humans the mRNA can be found in liver and large intestine but not in other organs *(Chu et al., 1993)*.

After the discovery of GSHPx-1 it was considered for many years that glutathione peroxidase activity in plasma was due to leakage of the enzyme from the liver and different

organs. Furthermore, plasma GSHPx activity increased with increasing selenium status and decreased with lower selenium status, consistent with the liver being postulated as the major source of the activity. Additionally, the plasma GSHPx activity had similar substrate specificities to GSHPx-1 *(Arthur, 2000)*. However, plasma GSHPx (GSHPx-3) did not react with antibodies to GSHPx-1 originally purified from RBC *(Takahashi and Cohen, 1986)* that would precipitate RBC or liver GSHPx-1. This led to attempts to purify GSHPx-3 from human plasma, work which showed that the protein is an isoenzyme of GSHPx with distinct properties. GSHPx-3 is a glycoprotein with an extracellular function *(Takahashi et al., 1987)*. The partial sequencing of the protein and then its cDNA confirmed that GSHPx-3 is distinct from GSHPx-1. The cDNA of GSHPx-3 has been sequenced for many species, including human, rat, mouse and bovine. These studies predict subunit molecular weights of approximately 23-25 kDa with between 40 and 50% homology with human GSHPx-1 *(Takahashi et al., 1990)*. The mRNA for GSHPx-3 is found predominantly in kidney *(Avissar et al., 1994a)*, heart, placenta, lung, gastrointestinal cells and thyroid *(Avissar et al., 1994b; Howie et al., 1995; Maser et al., 1994; Tham et al., 1998)*.

GSHPx-4 or phospholipid hydroperoxide GSHPx is the fourth selenium-containing GSHPx which has been characterised. This is a 20-22 kDa protein that contrasts with GSHPx-1 in that it is a monomer that can react with phospholipid hydroperoxide as substrate *(Ursini et al., 1995)*. There are many differences between GSHPx-4 and other GSHPxs. The major structural difference is that GSHPx-4 is a monomer in contrast to the tetrameric structure of other GSHPxs *(Schuckelt et al., 1991)*. GSHPx-4 can use hydrogen peroxide as substrate as well as a wide range of other lipid hydroperoxides. The fact that GSHPx-4 is a monomer may allow it to bind to the wider range of substrates than the tetrameric GSHPxs *(Maiorino et al., 1991)*. In contrast to GSHPx-1 and GSHPx-2, GSHPx-4 can also use a wide range of reducing substrates as well as glutathione *(Maiorino et al., 1995)*. Cloning GSHPx-4 showed it to be a polypeptide of 170 amino acids with a theoretical molecular weight of approximately 19 kDa. The sequence identity of GSHPx-4 is between 30-40% of GSHPx-1, dependent on the species being compared *(Sunde et al., 1993)*.

Some authors have argued, therefore, that GSHPx-4 is not a glutathione peroxidase. However, all the GSHPxs have a selenocysteine at the active site, which is successively oxidised and then reduced during catalytic cycles. Additionally, there are tryptophane and glutamine residues that are conserved in all the GSHPx *(Aumann et al., 1997)*. Thus, they

are clearly a family of similar enzymes. Like GSHPx-1, replacement of the selenocysteine at the active site of GSHPx-4 with cysteine dramatically decreases catalytic activity *(Maiorino et al., 1998)*. This catalytic activity with phospholipid hydroperoxides is stimulated by addition of detergents, which has led to belief that the enzyme is membrane associated *in vivo (Ursini et al., 1985)*.

3. 2. 3. Non-selenium-dependent GSHPx

The family of glutathione-S-transferases has many members which have GSHPx activity. This activity is against organic hydroperoxides, and thus when hydrogen peroxide is used as substrate, the activity is not measured. The transferases may still play an important role in protection against oxidative stress by mechanisms involving conjugation of aldehydes and other potentially oxidative compounds *(Hayes et al., 1999)*.

3. 2. 4. GSHPx-like protein

Some other proteins which are very similar to the seleno-GSHPxs have been identified:

An epididymis-specific GSHPx (GSHPx-5) has been demonstrated in rats, mice, pigs, monkeys and humans. Cloning these proteins shows them to be distinct from the other GSHPx, with approximately 60-70% identity to the GSHPx-1 coding region. A major difference is that cysteine is retained at the active site of GSHPx-5 *(Ghyselinck and Dufaure 1990; Ghyselinck et al. 1991; Hall et al., 1998 ; Vernet et al., 1996; 1997; Williams et al., 1998)*.

An odorant metabolising protein (GSHPx-6) has been sequenced and has 40% amino acid sequence identity to GSHPx-1, with cysteine in place of selenocysteine. It is found in the Bowman's gland of the olfactory system. There is still uncertainly as to its biological function *(Dear et al., 1991)*.

3. 2. 5. Hypothetical mechanism of action of glutathione peroxidase

The present view of mechanism of GSHPx is based on the following facts:

1. Lack of specificity with respect to the hydroperoxide *(Flohé et al., 1976)*.
2. High specificity for GSH *(Flohé et al., 1971a)*.
3. Selective inhibition by iodoacetate of the substrate-reduced enzyme only *(Flohé and Günzler, 1974)*.

4. Increased binding of *P*- chloromercuribenzoate by the enzyme on reduction by GSH *(Flohé et al., 1971b)*.
5. The ping-pong kinetics *(Flohé et al., 1972; Günzler et al., 1972 and Chiu et al.,1975)*.
6. The identification of a selenol as functional group *(Forstrom et al., 1978)*.
7. The reactivity of the enzyme- bound selenium with the physiological substrates *(Wendel et al., 1975)*.

The catalytic cycle can be formulated as follows (Fig. 3): a reduced form of the enzyme (E) reacts with a hydroperoxide in an uncomplicated bimolecular reaction. Neither the kinetics nor substrate-specificity studies indicate that an enzyme-substrate complex is involved in this catalytic step. The oxidized enzyme (F) then forms a complex with GSH. This complex, however, is rapidly transformed into a new intermediate (G) in an intramolecular reaction. Complex formation in the second step is supported by the high specificity of the enzyme for GSH, but not by the kinetic analysis. The intriguing observation that limiting Michaelis constants or limiting maximum velocities for GSH cannot be achieved despite high specificity, however, is compatible with the assumption that the formation of complex (F**.**GSH) is much slower than the intramolecular transformation into the intermediate G. In the last steps the second molecule of GSH regenerates E from the intermediate G, whereby a process analogous to step 2 has to be assumed. As to the chemical nature of the three enzyme forms, (E, F and G), the following ideas can be proposed: E most likely represents the enzyme containing a largely dissociated selenol function of the selenocysteine residue ($E\text{-}Se^{-}$). The selective inhibition of E by iodoacetate, which does not show the pH dependence typical for the reaction with SH groups *(Günzler 1974)*, strongly supports this assumption. The results of *(Wendel et al., 1975)* and the binding of an additional 4 mol of *P*- chloromercury-benzoate to the GSH-reduced enzyme *(Flohé et al., 1971c)* also point to a selenol function. Finally, preliminary X-ray crystallography has revealed that the enzyme-bound selenium is exposed at the surface of the molecule and is thus in an excellent position to react with various hydroperoxides.

In F the selenium may be oxidized to a selenic acid derivative (E-SeOH) *(Ganther et al., 1976)*. However, a mixed selenosulphide (RSe·SR') cannot be excluded at the moment as an alternative. Both forms would readily react with thiols to generate finally disulphides and selenols. The most likely intermediate in both types of reactions would be a mixed selenosulphide consisting of the enzyme-bound selenium and GSH (E-Se·SG). Such a compound may represent the kinetic entity G.

Glutathione peroxidase

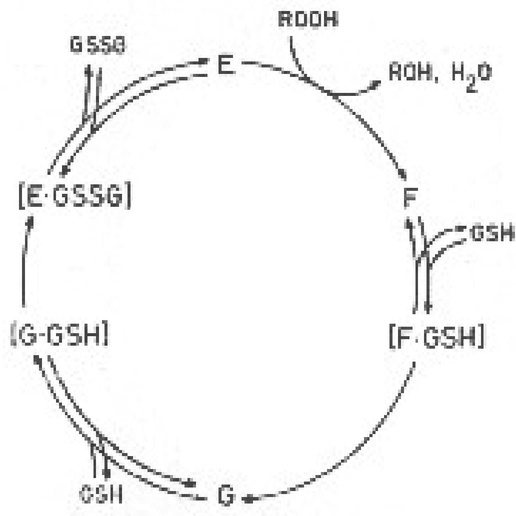


Fig. 3. Schematic representation of the GSH peroxidase reaction ***(Adapted from Flohé, 1978)***

3. 2. 6. Established function of GSHPx in living systems

a. Human red blood cells deficient in GSHPx are highly susceptible to pro-oxidative drug metabolites or xenobiotics. GSHPx deficiency results in a clinical condition very similar to favism, i.e. glucose-6-phosphate-dehydrogenase deficiency *(Necheles, 1974)*.

b. In the red blood cell, GSHPx scavenges most of the H_2O_2 generated in the cells and preventing degradation of the heme moiety, GSHPx plays unique role in protecting the red blood cell from endogenously generated H_2O_2, GSHPx has ability to react with H_2O_2 generated in close proximity to the red blood cell membrane in conjunction with the aut-oxidation of membrane-bound hemoglobin *(Nagababu et al., 2003)*.

c. Rat erythrocytes made deficient in GSHPx by means of a diet low in selenium are prone to peroxide-induced haemolysis *(Rotruck et al., 1972)*. These observations prove that GSHPx contributes essentially to the integrity of the red blood cell membrane.

d. Perfusion studies have demonstrated that exogenous hydroperoxides including H_2O_2 are metabolised by rat liver via GSHPx *(Sies et al., 1972, 1974)*. The hydroperoxides infused into the liver might to some degree mimic hydroperoxide originating endogenously outside the peroxisomal compartment. H_2O_2 generated within the peroxisomes by urate infusion usually results in compound I formation of catalase without the decrease of NADPH-dependent fluorescence and without the marked GSSG release typical for GSHPx function. These experiments were interpreted as showing that GSHPx is responsible for removing H_2O_2 only in those cell compartments which are low in or free of catalase.

e. In rat liver the compartments primarily protected by GSHPx are the cytosol and the mitochondrial matrix space, and the enzyme is hardly detectable in microsomes, nuclei and the peroxisomes, which probably contain the entire catalase of the cell *(Flohé and Schlegel, 1971)*.

f. GSHPx plays a key role in modulating the GSH/GSSG ratio and indirectly affects the NADP/NADPH quotient of the cell. The enzyme may thereby regulate multiple cellular functions such as cell division *(Kosower and Kosower, 1974, 1976)*, pentose-phosphate shunt *(Paniker et al., 1970; Egglestone and Krebs 1974 and Flohé 1976)*, gluconeogenesis *(Sies et al., 1974)*, mitochondrial oxidation of ∝-oxo-acids *(Sies and Moss, 1978)* and others *(Kosower and Kosower, 1974)*.

g. Some possible and/or probable roles of glutathione peroxidase itself that might be relevant to the prevention of chemical mutagenesis and the interaction with the arachidonic acid cascade leading to various prostaglandins *(Flohé, 1978)*.

h. Glutathione peroxidase have been demonstrated in human ovarian follicular fluid, as the activity was higher in follicles yielding oocytes that were subsequently fertilised than as compared to follicles with subsequently nonfertilized oocytes *(Paszkowski et al., 1995)*.

i. Glutathione peroxidase in oocytes is likely to improve fertilization rates in (*In vitro* fertilization) procedures and seems to protect the developing embryo from carcinogens and oxidative stress *(Knapen et al., 1999)*.

j. Se-GSHPx inhibited the initiating and promoting stages of chemical carcinogenesis *(Ip, 1981)*.

k. GSHPx reduces lipid hydroperoxides, potentially harmful prooxidants that may promote peroxidation of polyunsaturated fatty acids in phospholipids in biological membranes *(Flohé, 1999)*.

3. 2. 7. Prevention of lipid peroxidation by glutathione peroxidase

Glutathione peroxidase consistently prevents the oxidative breakdown of unsaturated lipids of biomembranes *in vitro*, and no reason to rule out the possibility that this enzymatic ability plays a part in the defence against oxidative damage of organisms living in aerobic conditions *(Flohé et al., 1976)*. This view is predominantly based on the following observations:

1. GSHPx can reduce hydroperoxy-fatty acid esters *(Little and O'Brien 1968)*.
2. Endogenous mitochondrial GSHPx prevents lipid peroxidation and irreversible high-amplitude swelling of rat liver mitochondria *(Flohé and Zimmermann 1970)*.
3. In isolated inner membranes of rat liver mitochondria purified GSHPx prevents the oxidative degradation of phospholipids and the concomitant formation of malonaldehyde *(Flohé and Zimmermann 1974)*.
4. Bovine blood GSHPx added to illuminated chloroplasts inhibits swelling and malonaldehyde formation *(Flohé and Menzel, 1971)*.
5. *In vivo* inhibition of GSHPx by repeated administration of cadmium salts results in an accumulation of degradation products of unsaturated lipids in rat testes *(Omaye et al., 1975)*.
6. Conditions requiring a high rate of lipid peroxide removal, such as the ingestion of lipid peroxides *(Reddy and Tappel, 1974)* or exposure to ozone *(Chow and Tappel, 1972)*, lead to increased GSHPx activity.

7. In rats deficient in selenium and consequently in GSHPx, lipid peroxidation can be detected *in vivo* by monitoring the evolution of ethane. The effect can be inhibited partially by selenium alone and more consistently by a combined treatment with selenium and α-tocopherol *(Hafeman and Hoekstra, 1976)*.

8. Low selenium and GSHPx levels were detected in Finnish children suffering from neuronal ceroid lipofuscinosis *(Westermarck, 1977)*.

In spite of this overwhelming though admittedly indirect evidence, the hypothesis that GSHPx acts directly on hydroperoxy groups of biomembrane lipids has been repeatedly questioned *(McCay et al.,1976 and Burk et al., 1978)*. It may be difficult to imagine that an enzyme which at least after conventional cell fractionation appears to be entirely soluble *(Flohé and Schlegel, 1971)* may have access to the hydrophobic lipid bilayers. The same reasoning, however, applied to a second type of GSHPx, which has recently been considered as a possible substitute for the seleno-enzyme in this special function *(Burk et al.,1978)*. Apart from being a poor GSH peroxidase, this selenium-independent enzyme is identical with ligandin *(Arias et al.,1976)* and glutathione transferase (EC 2.5.1.18; *Prohaska and Ganther, 1977)*, also catalyses the isomerisation of prostaglandin-endoperoxide (PGH_2) to PGE_2 *(Christ-Hazelhof et al.,1976)*. *McCay et al., (1976)* claimed that GSHPx possibly cannot reduce the hydroperoxides of membrane lipids directly, since they detected a lipid peroxide in a microsomal system and were unable to find a corresponding amount of hydroxy-lipid in the presence of glutathione peroxidase, although this enzyme, in contrast to catalase, prevented the oxidative lipid degradation as measured by the thiobarbituric acid reaction. To explain these findings the authors had to exhaust first the much-quoted Haber-Weiss cycle *(Haber and Weiss, 1934)* and secondly the argument that GSHPx is superior to catalase in destroying low concentrations of H_2O_2. Otherwise, according to some other authors, this interpretation cannot be accepted for several reasons:

(a) Malonaldehyde is not derived predominantly from lipid hydroperoxides but from cycle dialkyl peroxides *(Dahle et al.,1962)* which are not substrates of GSHPx.

(b) The lipid peroxide intermediate detected by *McCay et al., (1976)* was not necessarily a hydroperoxide, since the method of identification does not distinguish clearly between different types of peroxides (*Stahl,* 1958). The chromatographic behaviour of the lipid peroxide did not suggest the presence of a hydrophilic hydroperoxy group *(Tam and McCay, 1970)*.

(c) The rate constants for H_2O_2 removal of GSHPx and catalase *(Chance et al.,1952)* are rather close. Minor differences in the respective constants cannot account for an all-or-none difference in the biological effectiveness of the two enzymes under consideration.

The observations of *McCay et al., (1976)* are in good agreement with those of other studies on oxidative membrane damage. In the experiments with mitochondrial membranes GSHPx on a molar basis is also much more effective than catalase in preventing oxidative lipid degradation *(Flohé and Zimmermann, 1974)*. Similarly, oxidative haemolysis occurs in human red blood cells, which are deficient in GSHPx, but rich in catalase, while acatalasemic erythrocytes are not particularly prone to haemolysis *(Aebi and Suter, 1974)*. An explanation for the superiority of GSHPx in these systems which is consistent with the established enzymatic data must take into consideration the fact that the enzyme not only reduces H_2O_2 as fast as catalase but in addition other hydroperoxides. The nature of this hydroperoxide, which obviously initiates membrane destruction, still must be established, but a lipid hydroperoxide remain a likely candidate.

3. 3. Genetics of GSHPx enzyme activity

Several factors are known to affect the GSHPx activity including dietary selenium, age and sex, oestrus cycle, and environmental factors including exposure to ozone or ingestion of peroxidised lipids. Also there are some publications suggesting significant role for genetics in regulation of its activity. *Tucker and Kilgour (1970)* have shown that GSH concentration is controlled by a single pair of alleles and *Andrewartha (1978)* has reported a positive relation between GSH concentration and GSHPx activity. Such an association might explain the origin of the genetic differences.

3. 3. 1. Phenotypic variation of GSHPx enzyme activity in different animal species

There are numerous data about the phenotypic variations of GSHPx enzyme activity in different animal species. The actual activity of the enzyme depends on the rate of gene expression *(Condeell and Tappel, 1983, Takahashi et al., 1987)* and also affected by the amount of substrate, e. g. lipid peroxides *(Sies, 1986 and Sagara et al., 1998)*, selenium supply *(Rotruck et al., 1973)* and amino acid (methionine and cysteine) supply

(Wang et al., 1997). Among the above mentioned factors the genetic one has importance, but it is markedly modified by the environmental factors.

The presence of the enzyme activity in so many animal groups implies the widespread occurrence of genetic information for the specific assimilation of the selenium atom *(Smith and Shrift, 1979)*. GSHPx activity of animal tissues was studied by *Tappel et al. (1982)* and they found that total GSHPx activity of liver was the highest in the order: hamster greater than gerbil, mouse and rat approximately equal to rabbit. Considering the phylogenetic distribution of total GSHPx activity, the rodent limb of the phylogenetic tree has the highest activity. The regulation of GSHPx gene expression relevant to species difference was studied by *Toyoda et al. (1989)* and these results suggested that GSHPx activity in the cytosol of all guinea-pig tissues examined are extremely low as compared with those in mice and rats. The species difference of GSHPx activity observed in rodents might be due to incapability of gene transcription.

It is well-known that various animal species and various strains of mice show different degrees of susceptibility to oxidant gases such as nitrogen dioxide and ozone. However, the biochemical causes of the differences in susceptibility to oxidant gases between various animals still remain in question. Antioxidant protective enzymes were examined in the lung of four animal species exposed to a mixture of nitrogen dioxide and ozone for two weeks. Male mice, hamsters, rats and guinea pigs were used. The most characteristic change is the significant increase in GSHPx activity in hamsters and rats followed by mice and guinea pigs are genetically deficient in this enzyme (*Ichinose et al., 1988)*.

The activity of GSHPx in seminal plasma varied markedly among species. In boar and stallion the seminal plasma activity is absent, while the human and ram GSHPx activity are rather low. In contrast, bovine seminal plasma displays a high activity *(Saaranen et al., 1989)*.

To better define the species-specific antioxidant systems and to ascertain the influence of the intracellular redox status on the immune system of different animal species, *Chiaradia et al. (2002)* determined lymphocyte GSHPx activity in horse, sheep and dog. Sheep presente the highest GSHPx activity, dogs have the lowest and horses display intermediate values.

Species-specific expression of GSHPx has been found in fowl seminal plasma and spermatozoa *(Surai et al., 1998a; 1998b, 1998c)*. Studied in five avian species, the total GSHPx activity in seminal plasma is significantly higher in turkeys than in ducks and

geese. In contrast, waterfowl species; geese and ducks are characterised by the highest sperm GSHPx activity. Such high expression of GSHPx in duck semen is shown to be a compensatory mechanism to protect drake spermatozoa with high levels of PUFA and low vitamin E concentration against lipid peroxidation *(Surai et al., 2000)*. It is important to note that the Se-dependent form of GSHPx comprised between 77.7% (chicken) and 87.4% (guinea fowl) of total enzymatic activity. The enzyme is distributed between spermatozoa (40%) and seminal plasma (60%) *(Surai et al., 1998a)*.

3. 3. 2. Phenotypic variation of GSHPx enzyme activity within some animal species

Mice:

GSHPx activity is elevated in skeletal muscle of mice with genetic muscular dystrophy than the normal mice strain *(Bell and Draper, 1976)*.

Ichinose et al., (1982) examined the strain differences of mice in the susceptibility to nitrogen dioxide by measuring the activity of antioxidant enzymes including GSHPx and they observed genetic differences in the GSHPx activity among the different mice strains.

Schisler and Singh (1988) assessed the GSHPx activity in the liver, kidney, lung and blood of mice from seven strains at five age groups and they found that GSHPx activity was the highest in the liver followed by blood hemolysate, kidney and lung. Although activity was shown by statistical analysis to be not significantly different among the strains, age-associated, strain-specific changes in GSHPx activity were noted to be highly significant. No strain displays the same ageing profile in the four tissues studied.

Ragusa et al., (1996) examined the antioxidant enzyme status of extraocular, diaphragm and gastrocnemius muscles in control and dystrophic skeletal muscle strains. Their results revealed that in the control strain, both extraocular and diaphragm muscles have higher GSHPx activity than dystrophic strain.

Rat:

The Long Evans rat with cinnamon-like coat color (LEC) is a mutant strain that displays hereditary hepatitis with severe jaundice. About 4 months after birth most LEC rats suddenly exhibit severe hepatitis with a high mortality, while the residual small number of rats show chronic hepatitis without jaundice *(Sasaki et al., 1985)*. All of the surviving rats develop chronic hepatitis and about 12 months later, primary liver cancer frequently develops. *Ohhira et al. (1995)* studied the changes in the activity of GSHPx during the course of spontaneous hepatitis in LEC rats and they found that GSHPx activity in liver is lower in LEC rats than in controls throughout the observation periods.

Recent papers reported decreases in GSHPx activity in livers from genetically and spontanously hypertensive rats (SHR) as compared to control Wistar Kyoto (WKY) rats *(Bui et al., 1995; Vericel et al., 1994; Hong and Johnson, 1995 and Binda et al., 2001)*. Liver GSHPx activity was markedly influenced by strain difference of Wistar and Fisher rats, in particular, GSHPx activity in Wistar rats resulted in a significant increase after ten months of age and remained at this level till 23 months of age. However, GSHPx activity in the small intestin was not affected by strain difference *(Jang et al., 2001)*.

Pig:

Jørgensen et al., (1977) investigated a possible genetic influence on the RBC GSHPx activity in the population consisting of 14 litters of pigs kept in the same environment and given the same diet. Their results showed that, the variation between litters was significantly higher than the variation within single litter. The litter effect explained about 80% of the total variation found in RBC GSHPx activity. Genetic predisposition of pigs to hypo-and hyperselenemia was also reported *(Stowe and Miller, 1985)*. *Lingaas et al. (1991)* estimated the variation of Se and GSHPx activity in blood plasma in two breeds of pigs and they reported that there was no statistical difference between breeds. The estimates of heritability for Se and GSHPx activity were 0.41 and 0.47, respectively, which indicated that, it is possible to increase the levels of both Se and GSHPx in blood plasma of pigs by selection. This would reduce the risk of clinical Se deficiency in individual pigs, especially in association with Se deficient diets. Also, no significant differences were found for blood GSHPx activity among three breeds of pigs *(Lingaas et al., 1992)*.

Sheep:

Langlands et al., (1980) have reported a significant variation in the GSHPx activity both among the breeds of a grazing flock in Australia and among the sires within breeds, the latter resulting in an estimate of the heritability of GSHPx activity per gram haemoglobin of RBC haemolysate of 0.5. The activity of GSHPx in whole blood and the Se concentrations in whole blood and wool were measured in samples taken from three breeds, Scottish Blackface, Welsh Mountain and Finnish Landrace sheep on the same pastures. Finnish Landrace sheep had almost twice the GSHPx activity and blood Se concentration of the other two breeds *(Wiener et al., 1983)*. Evidence of genetic variation

in GSHPx activity within the Finnish Landrace breed of sheep has been reported by *Atroshi et al. (1981)*. They divided the sheep in two categories according to their erythrocyte GSHPx activity (greater or less than 1000 µkat/L), mating between low GSHPx activity sheep resulted in low GSHPx activity of their offspring as measured at three months of age. On the other hand mating between high GSHPx activity types resulted in both high and low types of offspring. These results support the hypothesis that, the GSHPx activity levels are controlled by a single pair of autosomal alleles, the gene for high GSHPx activity being dominant to that for low GSHPx activity. It appears that the inheritance of the erythrocyte GSHPx activity in Finn sheep is polygenic in nature *(Atroshi et al., 1981)*. However, in another study of GSHPx activity in a flock of grazing sheep, which comprised the progeny of six breeds of sire mated to Scottish Blackface dams, no breed differences were found *(Woolliams et al., 1983)*.

Cattle:

Langlands et al., (1980) studied the effect of genotype as a source of variation in GSHPx activity of whole blood from grazing cattle. They reported that there were significant differences among six genotypes of cattle for whole blood GSHPx activity. Heterosis affects partly at the gene expression level of whole blood GSHPx activity in cattle, the heterosis as a percentage of the mid-parent values ranged from -11.3 to 25.5% *(Langlands et al., 1980)*.

Chicken:

Hull and Scott (1976) reported that GSHPx activity was equivalent in both chick strains of muscular dystrophic and non-dystrophic in plasma and liver, but was significantly increased in dystrophic once. GSHPx activity was assayed in the superficial pectoral muscles of genetically dystrophic chickens (line 413) and their control (line 412) by *Mizuno (1984)* and he found that in dystrophic chickens GSHPx activity was significantly elevated at all stages of development studied, and their developmental time courses was quite different from those in the controls. Broiler chickens showed significantly lower blood GSHPx activity than Leghorn-type chickens *(Shen et al., 1992)*. The involvement of lipid peroxidation in the development of liver haemorrhages in layer

hen chickens was investigated in two White Leghorn strains of birds, a commercial layer strain and strain UCD-003, which is predisposed to the development of liver haemorrhages. Liver GSHPx activity and egg production in UCD-003 birds was lower than in the normal birds *(Wu and Squires, 1997)*.

Antioxidant enzyme activity in Pulmonary Hypertension Syndrome (PHS) in broilers was reported by *Iqbal et al. (2002)* and they found that lung mitochondria and liver GSHPx activity was elevated in broiler with PHS compared to healthy bird, higher GSHPx activity in PHS could be due to up-regulation of the expression of this enzyme and an important adaptive response to greater hydrogen peroxide production as a result of electron leakage from the respiratory chain *(Iqbal et al., 2001a)*. Also, lung mitochondria isolated from broilers selected for PHS resistance exhibited lower GSHPx activity compared to lung mitochondria from broilers that were not selected for PHS resistance and there were no differences in GSHPx activity in liver. Greater hydrogen peroxide production was observed in broilers that were not selected for PHS resistance than in selected lung mitochondria as a consequence of greater electron leakage from the respiratory chain *(Iqbal et al., 2001b)*. These findings, therefore indicate that broilers that were not selected for PHS resistance lung mitochondria experience an inherently greater degree of oxidative stress than do broilers that selected for PHS resistance lung mitochondria that would potentiate hydrogen peroxide formation. Lung mitochondria from broilers that were selected for PHS resistance birds exhibited higher GSHPx that would help in catabolising the greater hydrogen peroxide. Genetic resistance to PHS is associated with lower oxidative stress and improved mitochondrial function *(Iqbal et al., 2001 a, b)*.

However, no differences were found in the GSHPx activity in the liver of birds of two strains of single comb white leghorn chickens *(Squires and Wu, 1992)*.

Previous studies have indicated that growth responses of young chicks to severe uncomplicated nutritional deficiency of Se may involve an hereditary component. *Bunk and Combs (1981)* observed that Leghorn chicks fed an amino acid-based diet containing an exceedingly low amount of Se, but adequate with respect to all other known nutrients, showed considerable variation in effects on growth and survival. They found that although one-third of the population showed severely depressed growth with associated pancreatic exocrine dysfunction, an equal proportion was able to grow apparently normally. This observation was also made by *LaVronga and Combs (1982)*, who tested the hypothesis that variance in the growth response to severe Se deficiency is due to hereditary factor. Their results showed the feasibility of developing, through selective breeding, lines of Single

Comb White Leghorn chickens that differed in sensitivity to dietary Se-deficiency as measured by impairment in the growth of young chicks. Further, they showed that such line-related differences in growth were associated with analogous differences in methionine-methyl group oxidation rate, which suggests that a lesion in the metabolism of the sulphur-containing amino acids may be the site of hereditary involvement in the metabolic need for Se. Subsequent studies by *Halpin and Baker (1984)* found similar evidence of aberrant sulphur-amino acid metabolism in one meat-type strain of chicken but effects in a Leghorn strain or a crossbred strain. The extent to which the consequences of nutritional Se deficiency may differ among genotypes is of fundamental importance to understanding the role of Se in normal metabolism and of practical significance to poultry feeding particularly in parts of the world with endemic Se deficiency *(Cunningham et al., 1987)*.

3. 4. Effect of sex on GSHPx activity

Several studies have suggested that many enzyme activity in animal tissues are affected by sex. Sex differences 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 several hypotheses, which may provide a physiological basis for sex differences in Se concentration and GSHPx activity:

- Selenium distribution to tissues may be different for males and females because of the greater priority for Se by some tissues. For example, the testes of males have priority for Se during deficiency *(Behne et al., 1982),* whereas the female has no comparable tissue.
- Selenium deficiency appears to depress cytochrome P-450 activity in hepatocytes *(Burk et al., 1978),* but this effect of Se does not appear to be mediated by GSHPx *(Burk, 1983)*. Because cytochrome P-450 activity is greater in male rat hepatocytes than in females *(Schenkman et al., 1967; Montellano, 1986),* a larger amount of hepatic Se may be associated with non-GSHPx functions, such as cytochrome P-450. This may result in less selenium available for GSHPx, and thus lower GSHPx activity.
- Cytochrome P-450 has peroxidase activity *(Montellano, 1986)*. Higher activity of this system in male rats may result in lower need for GSHPx.
- The higher metabolic activity of males in general, resulting in a greater need for oxygen radical protection.

Males have greater plasma, kidney, cytosol and RBC GSHPx activity and Se concentration than females in rats *(Finley and Kincaid, 1991; Debski et al., 1992)*. Male rats have higher levels of glutathione peroxidase in myocardium than in females *(Barp et al., 2002)*. *Burk (1983)* stated that GSHPx activity decreased more quickly in Se-deficient livers of male than female rats.

GSHPx activity, however, is higher in female than in male rats liver in both Se-deficient and Se adequate diet *(Burk, 1983; Capel and Smallwood 1983; Igarashi et al., 1984; Debski et al., 1992; Prohaska and Sunde, 1993; Sachdev and Sunde, 2001)*. GSHPx activity is more than double in hepatic mitochondria from rats females than in those from males of the same age *(Borras et al., 2003)*.

However, *Capel and Smallwood (1983)* reported that no significant differences found in comparison of glutathione peroxidase activity of the blood and brain tissues of male and female rats.

In mice liver GSHPx activity is higher in females compared to males *(Prohaska and Sunde, 1993)*.

GSHPx activity in red blood cells is higher in females than in males, but greater in plasma and aorta in males of Japanese quail *(Godin et al., 1995)*.

Blood glutathione peroxidase activity in normal dromedary camels were assessed in the Canary Islands by *Corbera et al. (2001)* and they found that females are significantly higher blood glutathione peroxidase activity than males.

However, the sex have no apparent influence on GSHPx activity of the red blood cell *(Jørgensen et al., 1977)* and in whole blood *(Lingaas et al., 1991)* of pigs.

No available data concerning the sex effect on GSHPx activity in chickens were found in the literature.

3. 5. Effect of age on GSHPx activity

The life in an oxygen-rich environment has required the evolution of effective cellular strategies to detect and detoxify metabolites of molecular oxygen known as reactive oxygen species. The appropriate and inappropriate production of oxidants, together with the ability of organisms to respond to oxidative stress, is intricately connected to ageing and life span *(Toren and Nikki, 2000)*. Nearly a century ago it was noted that animals with higher metabolic rates often have shorter life spans. These observations led to the formation of the rate-of-living hypothesis, which states that the metabolic rate of a species ultimately determines its life expectancy. Initially, the

mechanistic link between metabolism and ageing was unknown. In the mid 50s, Denham Harman articulated a free-radical theory of ageing, speculating that endogenous oxygen radicals were generated in cells and resulted in a pattern of cumulative damage *(Harman, 1956)*. According to its free radical theory, ageing can be viewed as a process of irreversible changes associated with an accumulation or integration of free radical induced damages in the cell *(Harman, 1956)*. According to the free radical theory ageing proposes as consequence of the deleterious effects of oxygen free radicals produced during normal cellular metabolism *(Harman, 1988)*. Virtually all cellular components, including DNA, proteins and lipids are susceptible to oxidative modifications *(Stadman, 1981; Halliwell and Gutteridge 1984)*. Oxidative damage to cellular structure and function was shown to be associated with age-related diseases, such as atherosclerosis, muscular dystrophy, arthritis, diabetes, pulmonary dysfunction, various neurological disorders and cancer *(Yu, 1994)*.

Brown adipose tissue responds to physiological stimulation with high rates of mitochondrial O_2 consumption, and with high rates of lipid turnover, in rats. These are the most susceptible molecules to peroxidation. Thus, it is important to elucidate the changes in antioxidant defence and lipid peroxidation that occur in this tissue during the lifetime of the organism. It was shown that during the development from young to mature adult age quantitatively important increases of all the antioxidant enzymes, including GSHPx, take place in brown adipose tissue *(Lopez-Torres et al., 1991)*.

In red blood cells of rats GSHPx activity increased with age was reported by *Flohé and Zimmermann (1970),* while the opposite tendency was found by *Glass and Gershon (1984)*. In sheep, red blood cell glutathione peroxidase activity slightly decreased with age *(Atroshi and Sankari, 1981),* while increased as effect of ageing in porcine blood *(Jørgensen et al., 1977)*.

The GSHPx activity increased with age in liver of rats *(Gonzalez et al., 1995; Gunther et al., 1993; Jang et al, 1998 and Palomero et al., 2001),* but the opposite tendency was found in mice *(Muradian et al., 2002)*.

In the small intestine of rats the enzyme activity was not affected by age *(Jang et al, 1998)*.

In the rat brain, GSHPx activity after an initial fall increases steadily with age *(Brannan et al., 1981; Scarpa et al., 1987),* however, no significant age-related changes were found in the activity in any of the brain areas *(Vertechy et al., 1993)*.

In the lung of rats the enzyme activity increased with age *(Gumuslu et al., 2001)*.

In the kidney of Wistar rats glutathione peroxidase activity showed an increase with age *(Santa and Machado, 1986),* but decreased with age in mice *(Toshinai et al., 1977)*.

Testicular glutathione peroxidase activity was twice as high in 4-months old animals as in weanling rats *(Behne et al., 1986)*.

Yildiz et al. (2002) reported that GSHPx activity in uteri was lower in young female sheep as compared to adult ones.

In guinea pig, the activity of GSHPx decreased significantly with age in the mitochondrial fractions of cerebral cortex and hypothalamus *(Vohra et al., 2001)*.

Age-related changes in GSHPx activity in chickens have been considered closely. Red blood cell and blood plasma GSHPx activity decreased with age in Japanese quail *(Godin et al., 1995)*. Liver GSHPx activity increased reaching a maximum at age 20-30 days of age *(Kalytka and Donchenko, 1995)*. In contrast, GSHPx activity declined in the superficial pectoral muscles, blood and RBC during ageing from 1 week up to 4 months of age *(Mizuno, 1984; Cestnik, 1985; Khan et al., 1995)*. In the semen of meat type cockerels from 25 to 60 weeks of age showed that GSHPx activity decreases with age *(Kelso et al., 1996)*.

3. 6. Phenotypic correlation between GSHPx activity and production traits

The erythrocyte GSHPx activity analysed in a flock of Finn sheep showed a negative correlation with body weight, weight gain and wool production *(Atroshi and Sankari, 1981)*. It is proposed that the low glutathione peroxidase activity might represent an adaptation to low selenium intake. Similar results were obtained with chicken *(LaVronga and Combs, 1982; Shen et al., 1992),* pig *(Lingaas et al., 1991)* and rabbit *(Mézes et al., 1994a)*. Also, there are some investigations directed to the GSHPx activity as a possible selection criteria for rabbit breeding as demonstrated a slight negative phenotypic correlation between carcass traits and enzyme activity of erythrocytes in New Zealand White and Pannon White rabbits *(Virág et al., 1996)*.

In chicken, liver GSHPx activity was positively correlated with egg production *(Squires and Wu, 1992)*.

3. 7. GSHPx activity and embryonic development

Chick embryo tissues are characterised by high concentration of polyunsaturated fatty acids *(Noble and Cocchi, 1990)* and are very sensitive to lipid peroxidation *(Gaál et al., 1995)*. The development of avian embryo is dependent on aerobic metabolism and, in particular, features the β-oxidation of fatty acids derived from yolk lipids *(Noble and Cocchi, 1990)*. The rate of oxygen consumption increases dramatically from about the mid-period of the 21-day development, partly because of the growth of the embryo but tends to become relatively constant during the last few days before hatching *(Freeman and Vince, 1974; Vleck and Hoyt, 1991)*. Such increases in mitochondrial respiration and oxygen uptake are obligatory aspects of embryonic development, providing energy for tissue growth, transport of nutrients from the yolk, maintenance of the heartbeat and other essential functions. However, it is likely that these beneficial aspects may be accompanied by potentially harmful effects, because high rates of energy metabolism can lead to the production of reactive oxygen species and other free radicals, which can cause damage to cellular macromolecules *(Halliwell, 1994a, b)*. Defence from lipid peroxidation is thus a crucial task for the organism. The antioxidant system of the chick embryo consists of a combination of natural metabolites *(Gaál et al., 1995)* and antioxidant enzymes, glutathione peroxidase, superoxide dismutase and catalase *(Wilson et al., 1992)*. Antioxidant enzymes are a major cell defence against acute oxygen toxicity *(Harris, 1992)* and those are responsible for the detoxification of reactive oxygen species and preventing lipid peroxidation *(Gille and Sigler, 1995)*. The expression of their activity is regulated in tissue-specific fashion at the gene level *(Bermano et al., 1995; Tsan, 1997; Weiss et al., 1997)*. They are considered as important embryo protective enzymes during organogenesis, when increased exposure to oxidant-derived free radicals or inadequate systems for antioxidant defence could alter cellular response at critical points in development *(Fantel, 1996; Allen and Venkatraj, 1992)*.

Species difference in the resistance of embryonic fibroblasts against oxygen-induced growth inhibition was studied by *Yuan et al. (1995)*. The growth of fibroblasts, which were isolated from human, rabbit, rat, mouse, and chick embryos, was inhibited partially under 50% oxygen and nearly completely under 95% oxygen. There was species difference in the resistance of these cells against oxygen-induced growth inhibition. The extent of resistance was in the following order: chick > rat> human > rabbit ≅ mouse. The order of their ability to recover from oxygen-induced growth inhibition was similar to the above order of species. There was also species difference in their antioxidant enzyme

activity, including GSHPx activity, and reduced glutathione concentration. Chick cells, having the highest resistance against oxygen-induced growth inhibition, were at the lowest activity levels of antioxidant enzymes and at the highest concentration level of reduced glutathione. *El Mouatassim et al., (2000)* studied the genetic expression of glutathione peroxidase involved in mechanisms protecting embryos against reactive oxygen species in human and mouse oviducts. Their results suggest that different gene expression patterns of glutathione peroxidase of human and mouse which reflect the variation in the ability of embryos to develop *in vivo* and *in vitro.*

Breed difference in the activity of glutathione peroxidase activity were reported by *Cestnik, (1985)*. The investigations included Rhode Island Red and Prelux-Bro breeds. Blood glutathione peroxidase activity was investigated in chicken embryos of different days of incubation and in chicks immediately after hatching. Significant differences were found between the breeds being higher for Rhode Island Red. Correlation between the activity of glutathione peroxidase and body mass was significant, as well.

Comparison between the various embryo tissues showed that the highest GSHPx activity at all stages of development was present in the liver *(Surai, 1999a)*. It was found that GSHPx activity of liver increased through the time of embryonic development *(Surai, 1999a; Taniguchi, 1997; Gaál et al., 1995)*.

No data was available about the sex differences in GSHPx activity during embryonic development.

4. MATERIALS AND METHODS

4. 1. Animals

The breeds used in these experiments are Hungarian Indigenous, Transylvanian Naked Neck, New Hampshire and Plymouth Rock White maintained in the Institute for Small Animal Research, Gödöllő, Hungary.

The origin of Hungarian and Transylvanian chicken breeds is not known exactly. *Winkler (1921)* and *Bakoss (1931)* presumed that progenitors of these birds were brought into the Carpathian basin from Asia by the Hungarian conquerors at the end of the ninth century. This (ancient Hungarian) chicken later must have mixed with other breeds (mostly Oriental and Mediterranean types), which formed the different Hungarian chicken breeds as they are known today. During the centuries of their formation, these breeds have been well adapted to the special climatic conditions and farming systems of the country, which made them very precious in this part of Europe. Until the beginning of commercial chicken breeding in Hungary these breeds of chickens of different colors (white and partridge in the Great Hungarian Plain, yellow and speckled in Transdanubia, mostly white, black and speckled naked neck breeds in Transylvania) were bred. They were preferred here not only for their relatively good egg production, but mostly for their excellent meat quality coming from the (seeking habit) of these birds, scratching for food regardless of hot or cold weather. They became strong, resistant to diseases and the costs of keeping were very low.

4. 2. Experimental conditions

Birds were kept under semi-extensive circumstances, which is usual in rearing this type of chickens. All animals were clinically healthy and kept in the same environment and given the same diets (Table 1). The criteria mentioned above was important because some factors such as pathological conditions like enteritis *(Mézes et al, 1986)*, high peroxide level of feed *(Mézes et al, 1994b)* or elevated temperature *(Mézes et al, 1993)* have measurable effect on the enzyme activity. The effect of oxidised oil feeding on the relationship between GSHPx activity and production traits was described by *Mézes et al. (1996)*.

Fertilised eggs were obtained from a commercial hatchery incubated at 37.8 °C and 60 % relative humidity in a forced-draught incubator with automatic egg turning.

Table 1. Nutrient content of the experimental diets

Nutrient content	Grower	Layer
MEp, MJ/kg	12.60	12,00
Dry matter, %	87.70	89.60
Crude protein, %	19.50	17.20
Crude fat, %	7.30	5.20
Crude fiber, %	2.50	3.00
Methionine, %	0.50	0.41
Lysine, %	1.08	0.80
Selenium, mg/kg	0.20	0.18
Vitamin E, mg/kg	28.60	33.00
Peroxide value, meq/kg	6.80	7.50
Acid value, meq/kg	9.40	10.50

4. 3. Experimental design

4. 3. 1. Experiment 1: GSHPx activity in chicken embryo

Fertilised eggs from eight breeds:

1. Plymouth Rock White (PRW)
2. New Hampshire (NH)
3. Hungarian White (HW)
4. Hungarian Speckled (HS)
5. Naked Neck Plymouth (NNP)
6. Naked Neck New Hampshire (NNNH)
7. Transylvanian Naked Neck White (TNNW)
8. Transylvanian Naked Neck Black (TNNB)

At least 400 samples (ten eggs of each breed) were weighed and embryos were killed at 14th, 16th, 18th and 20th day of development. Liver was dissected and immediately frozen (-20 °C) until analysed. Also blood and liver samples were taken from one day-old chicken (80 samples, ten of each breed).

4. 3. 2. Experiment 2: Phenotypic variation of GSHPx activity in different breeds of different age groups, from 1-day till the age of highest egg production

Blood samples were collected from six breeds:

1. Plymouth Rock White (PRW)
2. Naked Neck Plymouth (NNP)
3. Naked Neck New Hampshire (NNNH)

4. Hungarian Speckled (HS)

5. Transylvanian Naked Neck White (TNNW)

6. Hungarian White (HW)

In total, 1080 blood samples (15 males and 15 females of each breed at each age) were collected at one day, 4, 8 and 12 weeks of age, at the age of sexual maturity (SM) laying the first egg of females and at the period of the highest egg production. Liver samples were taken at one day old chickens only.

4. 3. 3. Experiment 3: Phenotypic variation in the activity of GSHPx in two chicken breeds and their crosses

In a preliminary experiment some breeds of the same age were tested for GSHPx activity and it were chosen the breed showed the highest enzyme activity (New Hampshire) and the lowest (Transylvanian Naked Neck Black) and made two crosses (New Hampshire sires crossed Transylvanian Naked Neck Black dames) and a reciprocal one (New Hampshire dames crossed Transylvanian Naked Neck Black sires).

At least 600 blood samples (15 males and 15 females of each breed at each age) were collected. Blood samples were taken at one-day, 4, 8 and 12 weeks of age and at the age of sexual maturity (SM) laying the first egg of females, respectively. Liver samples were taken from one-day old chickens only.

4. 4. Production traits

Egg weight (EW) and body weight (BW) were measured at the same time with liver and blood samples. Egg production at the highest egg production period (HEP) was recorded for each genotype (Table 2).

Table 2. Egg production % of different breeds

Breed	PRW	NNP	NNNH	HS	TNNW	HW
Egg production %	57.5 0	61.79	80.25	65.07	58.35	52.86

4. 5. Blood and liver samples

1. Blood samples were collected into tubes containing EDTA-Na_2 (0.2 mol/l) as anticoagulant. Freshly collected blood samples were centrifuged (15 min, 2,500 rpm), plasma was removed and stored frozen (-20 ºC) until analysed.
2. Erythrocytes were washed three times with two-fold volume of physiological saline (0.65 % w/v NaCl), then haemolysed with nine-fold of their volume of redistilled water and by freezing (-20 ºC, 24 hours) and thawing (37 ºC, 30 min).
3. Liver samples were homogenised freshly before analyses with nine-fold amount of physiological saline and the 10,000 g supernatant fraction was used for the determination of enzyme activity.

4. 6. Biochemical methods

1- Glutathione peroxidase activity was measured using reduced glutathione and cumene-hydroperoxide co-substrates *(Lawrence and Burk, 1976)* and the oxidation of reduced glutathione measured by the method of *Sedlak and Lindsay (1968)*. The enzyme activity was expressed in units reflecting the oxidation of reduced glutathione in nmoles per minute at 25 °C and was related to the protein content.

2- Protein content of blood plasma and erythrocyte haemolysate were determined using biuret method (*Weichselbaum, 1946*) while of liver homogenate using Folin phenol reagent *(Lowry et al., 1951)*.

3- Sex determination was carried out from embryonic blood samples at 14th, 16th, 18th, 20th days of incubation and from chicks at day-old using RAPD-PCR protocol *(Hidas and Edvi, 2001)*. In this procedure a single RAPD primer provided among several amplified DNA fragments a well expressed W chromosome specific fragment, which is suitable for sexing of the chicks.

4. 7. Statistical analysis

Means and SD of egg weight, body weight, liver, blood plasma and red blood cell GSHPx activity were subjected to analysis using three-ways ANOVA with breed, age and sex as main effects, according to the following model:

$$Y_{ijk}= M+G_i+A_j+S_k+(GA)_{ij}+(GS)_{ik}+(AS)_{jk}+(GAS)_{ijk}+e_{ijk}$$

Where: M= the common mean, $G_{i=}$ the effect of the ith breed; A_j= the effect of the jth age; S_k= the effect of the kth sex; $(GA)_{ij}$= the effect of interaction of the ith breed with the jth age;

$(GS)_{ik}$= the effect of interaction of the ith breed with the kth sex; $(AS)_{jk}$= the effect of interaction of the jth age with k^{th} sex; $(GAS)_{ijk}$= the effect of interaction of the ith breed with the jth age with the kth sex; e_{ijk}= random error term, using the GLM procedure of SPSS program *(SPSS for Windows, 1999)*.

Means and SD of body weight, blood plasma and red blood cell GSHPx activity in Experiment 1 were subjected to analysis using two-ways ANOVA with breed and sex as main effects, according to the following model:

Y_{ijk}= $M+G_i+S_k+(GS)_{ik}+e_{ik}$

Where: M= the common mean, $G_{i=}$ the effect of the ith breed; S_k= the effect of the kth sex: $(GS)_{ik}$= the effect of interaction of the ith breed with the kth sex; e_{ik}= random error term, using the GLM procedure of SPSS program.

Liver GSHPx activity was analysed by one-way ANOVA with breed as a main effect using one-way ANOVA of SPSS program.

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 the difference between the cross and midparent means. Reciprocal effect is the difference between the crosses of two parental breeds in which their roles as male or female parents are reversed.

Correlation analyses were performed by using the CORR procedure from SPSS. Spearman correlation coefficients (r_s) were used.

5. RESULTS

5. 1. Experiment 1: GSHPx activity in chicken embryo

Information concerning antioxidant enzymes in the chick embryos is very limited. The aim of this experiment was to study the effect of breed and sex on liver, RBC and blood plasma GSHPx activity in chicken embryo at 14th, 16th, 18th, 20th days of incubation and day-old chicks, and its correlation with egg weight and body weight at day-old age which has not been reported before in detail in chicken.

5. 1. 1. Phenotypic variation of GSHPx activity of embryo liver

Phenotypic variations of GSHPx activity of liver homogenate in different breeds are summarised in Table 3. Regarding the breed effect, significant differences were found among the breeds showing the highest enzyme activity in TNNW and the lowest in PRW breed (Fig. 4).

TNNW breed showed the highest liver enzyme activity and TNNB the lowest for males. TNNB breed showed the highest liver homogenate enzyme activity and PRW the lowest for females (Table 3).

Breed and sex interaction showed that GSHPx activity was higher in females than in males in all breeds except PRW (Table 3).

Sex had a significant influence on liver GSHPx activity, females having higher enzyme activity than males as shown in Table 3.

Concerning the effect of age, it can be seen that liver enzyme activity was significantly influenced by age as shown in Table 4. The enzyme activity decreased with age (Fig. 5).

Regarding breed and age interaction, variation of liver GSHPx activity showed a mixed pattern of increase and decrease depending on breeds (Table 4). There were significant differences in the GSHPx activity of liver among breeds in all age groups. These differences were not consequent in different age groups (Fig. 6). The most significant differences among breeds were observed at 16th day of incubation (Table 4).

Age and sex interaction showed that females having higher activity than males in all age groups (Fig. 5). However, these differences were significant at 14th day of incubation only.

Breed, age and sex interaction was statistically significant. The differences between males and females in different age groups have a wide variation among breeds (Table 4).

Table 3. Liver homogenate 10,000 g supernatant, GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	Liver GSHPx activity		
	Male	**Female**	**M+F**
NNP	1.51± 0.56 ^{a}	1.75± 0.58 ^{ab}	1.66± 0.58 ^{ab}
NNNH	1.54± 0.53 ^{ab}	1.73± 0.72 ^{ab}	1.63± 0.63 ^{ab}
HW	1.73± 0.42 ^{b}	1.78± 0.57 ^{ab}	1.75± 0.51 ^{ab}
HS	1.62± 0.41 ^{ab}	1.80± 0.61 ^{b}	1.74± 0.55 ^{ab}
PRW	1.65± 0.40 ^{ab}	1.59± 0.42 ^{a}	1.61± 0.41 ^{a}
NH	1.71± 0.49 ^{b}	1.77± 0.55 ^{ab}	1.74± 0.52 ^{ab}
TNNB	1.42± 0.59 ^{a}	1.84± 0.61^{b}	1.70± 0.63^{ab}
TNNW	1.78± 0.56 ^{ab}	1.79± 0.45 ^{ab}	1.78± 0.49 ^{b}
Sex effect	1.63± 0.50 ^{A}	1.76± 0.56 ^{B}	

$^{a, b}$ Means marked with different letters for each column are significantly different ($P < 0.05$).

$^{A, B}$ Significant differences between males and females ($P < 0.05$).

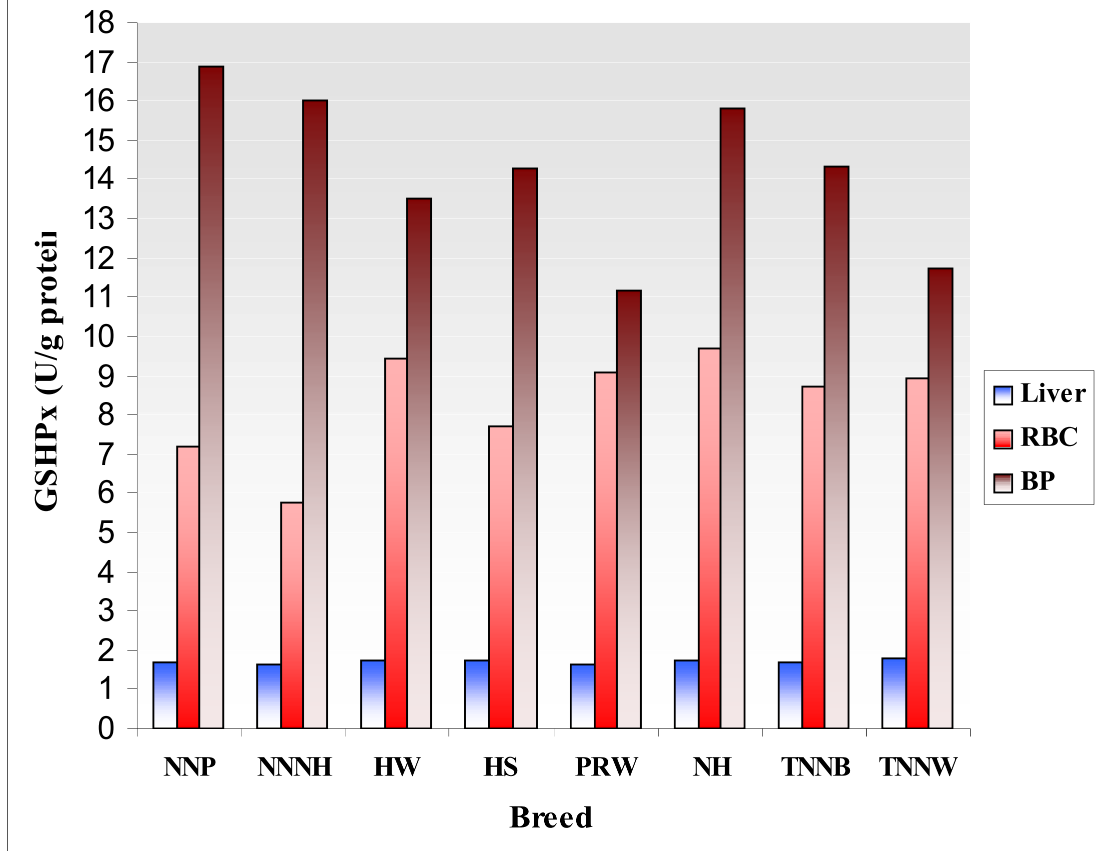


Fig. 4. Liver, RBC and PB GSHPx activities of different breeds
GSHPx (U/g protei
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
NNP
NNNH
HW
HS
PRW
NH
TNNB
TNNW
Breed
Liver
RBC
BP

Table 4. Liver homogenate 10,000 g supernatant, GSHPx activity (U/g protein) of different breeds sex and age groups (mean ± SD)

Breed	14th	16th	18th	20th	21th
			days of incubation		
NNP (M)	2.45±0.43	1.84±0.32	1.35±0.37	1.14±0.32	1.21±0.29
NNP (F)	2.55±0.51	1.54±0.51	1.59±0.34	1.40±0.18	1.53±0.27
NNP (M+F)	2.52±0.46 ^{ab}	1.63±0.46 ^{ab}	1.52±0.35 ^{ab}	1.30±0.27 ^{a}	1.34±0.32 ^{ab}
NNNH (M)	2.35±0.36 $^{\mathit{a}}$	1.53±0.33	1.37±0.39	1.43±0.25	1.06±0.35
NNNH (F)	2.84±0.20 $^{\mathit{b}}$	1.80±0.50	0.84±0.36	1.52±0.24	1.33±0.26
NNNH (M+F)	2.60±0.34 ^{b}	1.64±0.40 ^{ab}	1.21±0.44 ^{a}	1.48±0.23 ^{a}	1.19±0.32 ^{a}
HW (M)	2.13±0.41	1.99±0.12	1.58±0.25	1.32±0.42	1.69±0.20
HW (F)	2.51±0.57	2.01±0.39	1.31±0.42	1.42±0.37	1.61±0.01
HW (M+F)	2.32±0.51^{ab}	2.01±0.32 ^{c}	1.42±0.37 ^{ab}	1.37±0.38 ^{a}	1.65±0.14 ^{b}
HS (M)	2.37±0.22	1.92±0.01	1.65±0.15	1.41±0.31	1.41±0.36
HS (F)	2.48±0.37	1.81±0.51	1.39±0.63	1.53±0.28	1.56±0.43
HS (M+F)	2.46±0.34 ^{ab}	1.83±0.45^{abc}	1.47±0.53 ^{ab}	1.45±0.29 ^{a}	1.50±0.39 ^{ab}
PRW (M)	2.14±0.25	1.44±0.01	1.44±0.36	1.44±0.30	1.45±0.12
PRW (F)	2.17±0.10	1.39±0.39	1.58±0.43	1.50±0.39	1.51±0.35
PRW (M+F)	2.16±0.20 ^{a}	1.41±0.32 ^{a}	1.54±0.39 ^{ab}	1.47±0.33 ^{a}	1.50±0.29 ^{ab}
NH (M)	2.45±0.23	1.86±0.46	1.33±0.23	1.39±0.30	1.68±0.22 $^{\mathit{b}}$
NH (F)	2.50±0.22	1.95±0.32	1.74±0.33	1.50±0.39	1.19±0.37 $^{\mathit{a}}$
NH (M+F)	2.47±0.21^{ab}	1.90±0.38 ^{bc}	1.41±0.29 ^{ab}	1.45±0.30 ^{a}	1.44±0.39 ^{ab}
TNNB (M)	2.64±0.20	1.43±0.00	1.12±0.46	1.06±0.30 $^{\mathit{a}}$	1.47±0.40
TNNB (F)	2.64±0.57	1.72±0.42	1.63±0.31	1.50±0.46 $^{\mathit{b}}$	1.37±0.23
TNNB (M+F)	2.64±0.51^{b}	1.69±0.04 ^{abc}	1.53±0.38 ^{ab}	1.24±0.41 ^{a}	1.42±0.31 ^{ab}
TNNW (M)	2.45±0.30	1.64±0.45	1.63±0.01	1.37±0.55	1.63±0.19
TNNW (F)	2.43±0.45	1.80±0.36	1.80±0.35	1.49±0.30	1.50±0.24
TNNW (M+F)	2.44±0.36 ^{ab}	1.72±0.39^{abc}	1.78±0.33 ^{b}	1.43±0.42 ^{a}	1.54±0.22 ^{b}
Sex effect					
Male	2.34±0.34$^{\mathit{A}}$	1.71±0.36	1.41±0.31	1.32±0.35	1.44±0.34
Female	2.53±0.43 $^{\mathit{B}}$	1.74±0.45	1.53±0.45	1.48±0.29	1.46±0.30
Age effect	2.45±0.40 $^{\mathbf{c}}$	1.73±0.42 $^{\mathbf{b}}$	1.49±0.40 $^{\mathbf{a}}$	1.40±0.33 $^{\mathbf{a}}$	1.45±0.3$^{\mathbf{a}}$

$^{a,\ b}$ Means marked with different letters within each column are significantly different (P < 0.05).

$^{\mathit{a,b}}$ Significant differences within the breed between males and females (P < 0.05).

$^{\mathit{A,\ B}}$ Significant differences within the age between males and females (P < 0.05).

$^{\mathbf{a,b}}$ Significant differences within the age (P < 0.05).

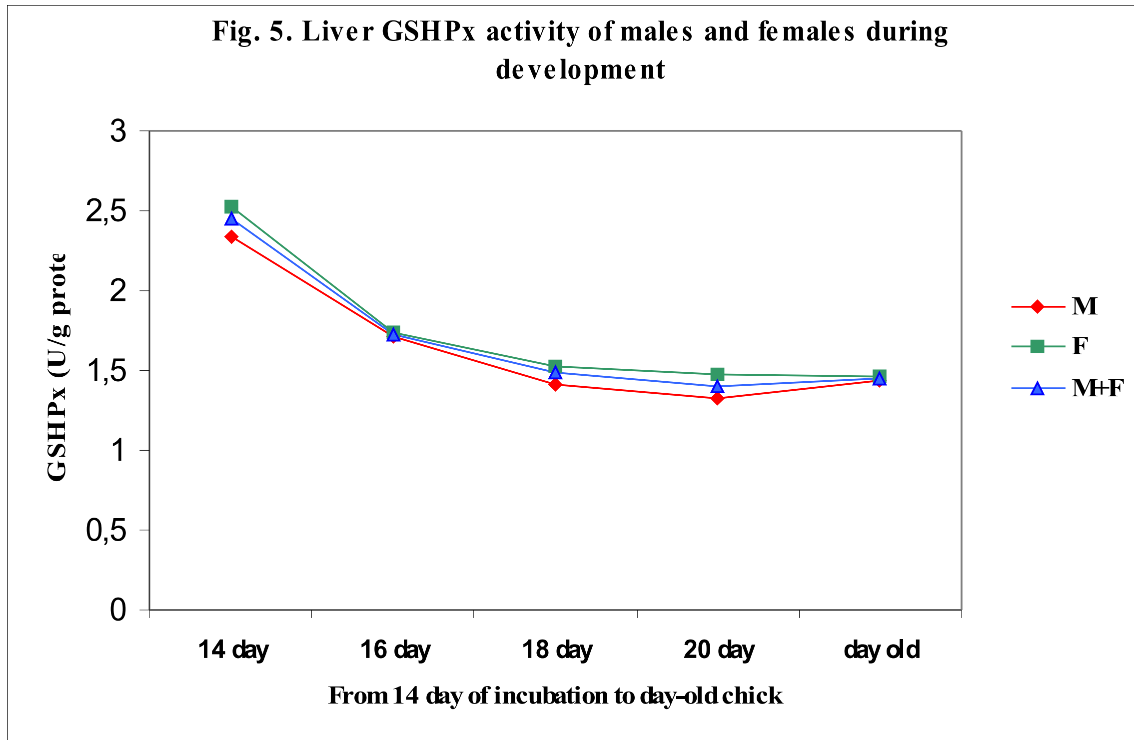


Fig. 5. Liver GSHPx activity of males and females during development
GSHPx (U/g prote
3
2,5
2
1,5
1
0,5
0
14 day
16 day
18 day
20 day
day old
From 14 day of incubation to day-old chick
M
F
M+F

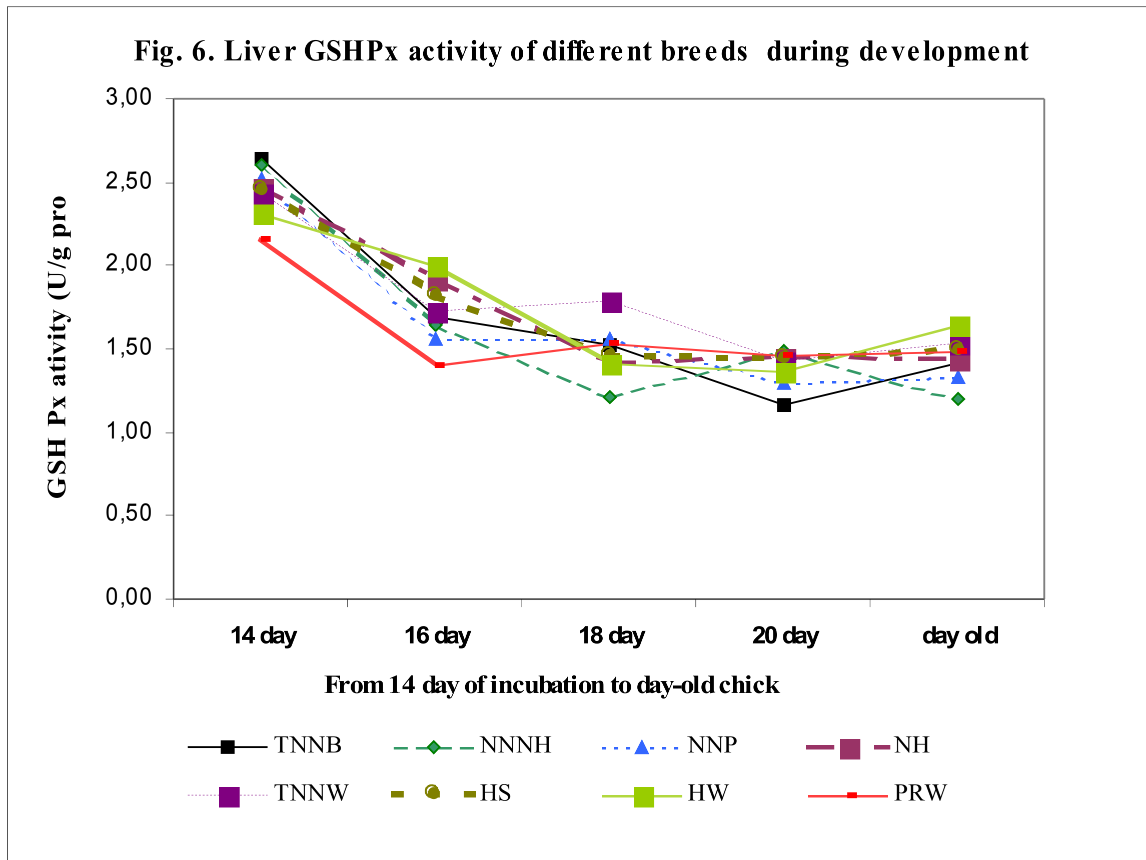


Fig. 6. Liver GSHPx activity of different breeds during development
GSH Px ativity (U/g pro
3,00
2,50
2,00
1,50
1,00
0,50
0,00
14 day
16 day
18 day
20 day
day old
From 14 day of incubation to day-old chick
TNNB
NNNH
NNP
NH
TNNW
HS
HW
PRW

5. 1. 2. Phenotypic variation of GSHPx activity of RBC

Phenotypic variations of the GSHPx activity of RBC in different breeds are given in Table 5. Regarding breed effect, significant differences were found among the breeds showing the highest enzyme activity in NH and the lowest in NNNH breed as shown in Fig. 4. The same tendency was found in males. For females HW breed showed the highest RBC enzyme activity and NNNH the lowest.

The GSHPx activity were higher in males than in females in NNP, HS, NH and TNNB breeds and higher in females than in males in NNNH, HW, PRW and TNNW breeds (Table 5).

Sex effect was not significant in the case of RBC GSHPx activity, however females had higher GSHPx activity than males (Table 5).

Table 5. Red blood cell GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	RBC GSHPx activity		
	Male	Female	M+F
NNP	7.28± 2.21 ^{ab}	7.11± 2.21 ^{ab}	7.21± 2.23 ^{ab}
NNNH	5.39± 2.52 ^{a}	6.15± 0.74 ^{a}	5.77± 1.80 ^{a}
HW	9.06± 2.40 ^{ab}	9.82± 3.19 ^{b}	9.44± 2.69 ^{b}
HS	8.51± 3.71 ^{ab}	7.15± 2.79 ^{ab}	7.69± 3.06 ^{ab}
PRW	8.86± 1.78 ^{ab}	9.15± 2.41 ^{ab}	9.06± 2.15 ^{b}
NH	9.75± 2.25 ^{b}	9.64± 2.63 ^{ab}	9.69± 2.31 ^{b}
TNNB	9.35± 4.47 ^{ab}	7.62± 0.47 ^{ab}	8.70± 3.50 ^{b}
TNNW	7.27± 0.25 ^{ab}	9.63± 3.41 ^{ab}	8.93± 3.01 ^{b}
Sex effect	8.16± 2.89 $^{N.S}$	8.42± 2.75	

$^{a, b}$ Means marked with different letters for each column are significantly different ($P < 0.05$).

$^{N.S}$ No significant differences between males and females ($P < 0.05$).

5. 1. 3. Phenotypic variation of GSHPx activity of blood plasma

Phenotypic variations of GSHPx activity of blood plasma in different breeds are summarised in Table 6. Concerning breed effect, significant differences were found among the breeds showing the highest enzyme activity in NNP and the lowest in PRW breed (Fig. 4). The same tendency was found in males. Otherwise NNNH showed the highest and PRW had the lowest blood plasma enzyme activity in females.

Breed and sex interaction showed that males had higher blood plasma enzyme activity than females in NNP, PRW, NH and TNNW breeds, while females showed higher enzyme activity than males in NNNH, HW, HS and TNNB breeds (Table 6).

Sex had no significant influence on blood plasma GSHPx activity, however, females showed higher activity than males (Table 6).

Table 6. Blood plasma GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	Blood plasma GSHPx activity		
	Male	**Female**	**M+F**
NNP	17.49± 3.50 ^{c}	15.97± 3.01 ^{bc}	16.88± 3.23 ^{d}
NNNH	15.59± 2.48 ^{b}	16.43± 2.55 ^{c}	16.01± 2.42 ^{cd}
HW	12.07± 2.99 ^{ab}	14.98± 3.59 ^{abc}	13.52± 3.47 ^{abc}
HS	11.87± 2.20 ^{a}	15.90± 3.26 ^{ab}	14.29± 3.44 ^{bcd}
PRW	11.65± 3.89 ^{a}	10.94± 1.00 ^{a}	11.15± 2.04 ^{a}
NH	16.07± 1.09 ^{bc}	15.55± 1.71 ^{bc}	15.81± 1.38 ^{cd}
TNNB	13.85± 1.89 ^{ab}	16.13± 2.01 ^{bc}	14.99± 2.20 ^{cd}
TNNW	12.35± 2.70 ^{ab}	11.47± 4.15 ^{ab}	11.74± 3.64 ^{ab}
Sex effect	14.23± 3.24 $^{N.S}$	14.35± 3.43	

^{a,b} Means marked with different letters for each column are significantly different ($P < 0.05$).

$^{N.S}$ No significant differences between males and females ($P < 0.05$).

5. 1. 4. Phenotypic variation of egg weight

Average egg weight are given in Table 7. Significant differences in the egg weight were found among the eight breeds during incubation period. NNP breed had the highest egg weight at 14th and 20th day of incubation, while NNNH had the highest egg weight at 16th and 18th day of incubation. The TNNW had the lowest egg weight during the whole period of incubation (Table 7).

Table 7. Egg weight (g) of different breeds, sex and age groups (mean ± SD)

Breed	14th	16th	18th	20th
		days of incubation		
NNP (M)	59.09±4.26	52.62±1.25	57.25±5.97	55.18±5.92
NNP (F)	56.82±3.98	52.60±2.36	53.71±3.64	55.08±3.00
NNP (M+F)	57.50±3.97 ^{d}	52.61±2.01 ^{c}	54.77±4.44 ^{e}	55.12±4.09 ^{f}
NNNH (M)	56.42±2.70	54.34±4.20	55.22±4.67	53.48±4.77
NNNH (F)	55.96±5.55	56.17±3.29	55.30±4.79	52.35±1.60
NNNH (M+F)	56.19±4.13 ^{cd}	55.07±3.78 ^{c}	55.24±4.43 ^{e}	52.80.3.06 ^{ef}
HW (M)	49.22±3.18 $^{\mathit{b}}$	45.48±3.01	47.08±2.19	44.38±1.32
HW (F)	42.96±2.59 $^{\mathit{a}}$	45.12±4.14	46.97±4.87	42.87±5.24
HW (M+F)	46.09±4.28 ^{a}	45.23±3.67 ^{ab}	47.01±3.84 ^{abc}	43.63±3.69 ^{b}
HS (M)	52.67±2.30	57.21±0,01	51.75±2.06	49.61±3.06
HS (F)	55.18±4.36	52.16±2.4	49.53±1.64	47.57±4.64
HS (M+F)	54.67±4.67	53.17±3.01	50.19±2.00 ^{bcd}	48.79±3.67 ^{cd}
PRW (M)	53.03±6.14	49.58±4.16	47.35±4.57	51.87±4.92
PRW (F)	53.75±4.22	53.96±3.97	52.58±5.59	50.97±3.35
PRW (M+F)	53.32±5.20 ^{bc}	52.65±4.34 ^{c}	51.01±5.64 ^{cd}	51.42±3.99 ^{de}
NH (M)	56.56±2.89	53.27±3.68	52.81±2.59	54.49±3.32
NH (F)	56.91±5.19	54.67±3.17	50.74±2.28	53.08±5.07
NH (M+F)	56.74±3.96 ^{cd}	53.97±3.32 ^{c}	52.40±2.56 ^{de}	53.64±4.30 ^{ef}
TNNB (M)	51.57±3.53	50.89±0.01	48.99±0.76	46.08±2.28
TNNB (F)	49.75±3.54	46.30±3.59	47.24±3.11	45.29±3.50
TNNB (M+F)	50.11±3.43 ^{b}	46.76±3.68 ^{b}	47.59±2.85 ^{abc}	45.77±2.67 ^{bc}
TNNW (M)	44.02±2.38	43.51±3.96	46.37±0.01	40.62±3.08
TNNW (F)	46.93±2.34	42.71±4.55	44.43±3.39	39.28±4.29
TNNW (M+F)	45.48±2.70 ^{a}	43.11±4.04 ^{a}	44.62±3.25 ^{a}	39.95±3.59 ^{a}
Sex effect				
Male	52.58±5.80	50.66±5.49	51.96±4.78	49.00±5.91
Female	52.47±6.03	50.14±5.50	49.34±5.03	48.78±6.85

$^{a, b}$ Means marked with different letters within each column are significantly different ($P < 0.05$).
$^{\mathit{a,b}}$ Significant differences within the breed between males and females ($P < 0.05$).

5. 1. 5. Phenotypic variation of body weight of day old chicken

Average body weight of different breeds are presented in Table 8. Regarding the breed effect, significant differences were found among breeds, NNNH had the highest and TNNW had the lowest body weight and the same tendency was found in males. In the case of females NNP had the highest and TNNW had the lowest body weight. Females had higher body weight than males in all breeds except in NNNH and PRW.

Sex effect showed that, females had higher body weight but this different was not significant.

Table 8. Body weight (g) of different breeds and sex of day-old chicken (mean ± SD)

Breed	Body weight		
	Male	Female	M+F
NNP	38.27±4.78 [def]	42.33±2.89 [f]	39.89±4.46 [cde]
NNNH	43.13 ±2.41 [f]	40.22 ±2.47 [ef]	41.68±2.77 [e]
HW	33.27±3.21 [abc]	35.47±3.18 [abc]	34.37±3.23 [ab]
HS	36.87±3.33 [bcd]	37.90±1.90 [cd]	37.48±2.45 [bcd]
PRW	39.29±1.39 [def]	38.57±3.56 [def]	38.79±3.00 [cde]
NH	38.98±5.36 [def]	41.90±4.04 [ef]	40.44±4.73 [de]
TNNB	35.21±3.02 [bcd]	38.85±2.67 [def]	37.03±3.30 [bc]
TNNW	30.31±1.78 [a]	32.63±2.89 [a]	31.94±2.75 [a]
Sex effect	37.19±4.86 [N. S]	38.12±4.16	

[a, b] Means marked with different letters for each column are significantly different ($P < 0.05$).
[N. S] No significant differences between males and females ($P < 0.05$).

5. 1. 6. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity and egg weight and body weight

Rank order phenotypic correlations between liver, RBC and blood plasma GSHPx activity and egg weight and body weight are summarised in Table 9. Negative correlations were found between liver GSHPx activity at 14th and 20th day of incubation and egg weight (EW) at all stages of development and for day old body weight (Table 9). While positive correlations were found between liver GSHPx activity at 16th and 18th day of incubation and in day-old chicken and EW at all stages of development. Even similar correlations were shown for day old body weight (BW) as shown in Table 9.

Negative correlations were also found between GSHPx activity in blood plasma and EW at 14th, 16th, 18th and 20th day of incubation and BW of day-old chicken (Table 9). Otherwise positive correlations were found between GSHPx activity in RBC and egg weight at 14th, 16th, 18th and 20th day of incubation and BW of day-old chicken as shown in Table 9.

Table 9. Rank order phenotypic correlation between GSHPx activity in liver, RBC haemolysate, blood plasma and egg weight (EW) and body weight (BW) of different age groups of chicken

GSHPx activity	EW 14	EW 16	EW 18	EW 20	BW 1
Liver					
14- day	-0.333	-0.190	-0.357	-0.286	-0.333
16- day	0.286	0.167	0.476	0.405	0.357
18-day	0.310	0.690	0.429	0.262	0.548
20-day	-0.238	-0.714*	-0.476	-0.286	-0.571
day-old	0.714*	0.595	0.833*	0.738*	0.786*
RBC-day old	0.333	0.262	0.452	0.262	0.310
BP-day old	-0.786*	-0.476	-0.714*	-0.714*	-0.667

* Correlation is significant at P≤ 0.05 level.

5. 1. 7. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity

Significant negative correlation was found between liver enzyme activity at 14th day of incubation with liver (-0.76; $p \leq 0.01$) and RBC (-0.52; $p \leq 0.05$) enzyme activity of day-old chicken and body weight (-0.33; $p \leq 0.05$), while significant positive correlation was found with blood plasma enzyme activity (0.76; $p \leq 0.01$).

5. 2. Experiment 2: Phenotypic variation of GSHPx activity in different breeds of different age groups, from day-old to the age of highest egg production

The objective of this experiment was to collect information on the possible genetic background of liver, RBC and blood plasma GSHPx activity in different breeds of chicken, and its correlation with some production traits, age and sex.

5. 2. 1. Phenotypic variation of GSHPx activity of liver homogenate

Phenotypic variations of GSHPx activity of liver homogenate in different breeds are summarised in Table 10. Significant differences were found among the breeds showing the highest enzyme activity in HW and the lowest in NNP breed (Fig. 7).

Table 10. Liver homogenate 10,000 g supernatant, GSHPx activity (U/g protein) of different breeds (mean ± SD)

Breed	Liver GSHPx activity
PRW	1.25±0.12 ^{ab}
NNP	1.21±0.05 ^{a}
NNNH	1.53±0.06 ^{c}
HS	1.49±0.09 ^{c}
TNNW	1.35±0.13 ^{b}
HW	1.61±0.14 ^{c}

Means marked with different letters are significantly different ($P < 0.05$).

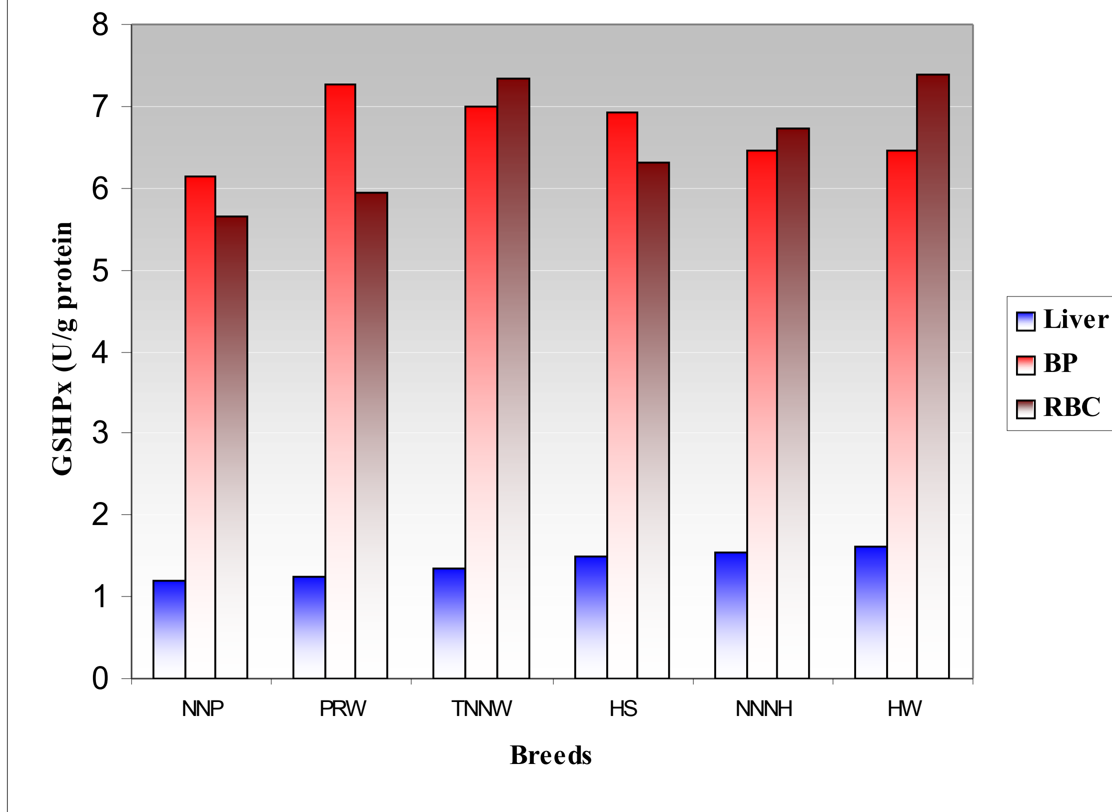


Fig. 7. Liver, RBC and blood plasma GSHPx activities of different breeds
GSHPx (U/g protein
8
7
6
5
4
3
2
1
0
NNP
PRW
TNNW
HS
NNNH
HW
Breeds
Liver
BP
RBC

5. 2. 2. Phenotypic variation of GSHPx activity of RBC

Phenotypic variations of GSHPx activity of RBC in different breeds are given in Table 11. Concerning breed effect, HW breed had the highest RBC GSHPx activity and NNP had the lowest (Fig. 7). The same tendency was found for females while, TNNW breed showed the highest RBC enzyme activity for males.

The GSHPx activity was higher in males than in females in all breeds except HW (Table 11).

Sex had a significant influence on RBC GSHPx activity, males having higher values than females as shown in Table 11.

Table 11. Red blood cell GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	RBC GSHPx activity		
	Male	**Female**	**M+F**
PRW	6.40±2.5 ^{b}	5.49±2.1 ^{a}	5.94±2.3 ^{ab}
NNP	5.83±2.3 ^{a}	5.48±2.0 ^{a}	5.66±2.2 ^{a}
NNNH	7.10±2.4 ^{c}	6.39±2.7 ^{b}	6.74±2.6 ^{c}
HS	6.50±2.4 ^{b}	6.11±2.2 ^{b}	6.30±2.3 ^{b}
TNNW	7.42±2.7 ^{c}	7.27±3.5 ^{c}	7.34±3.1 ^{d}
HW	7.29±2,6 ^{c}	7.50±2.9 ^{c}	7.39±2.7 ^{d}
Sex effect	6.75±2.5 ^{B}	6.37±2.7 ^{A}	

$^{a, b}$ Means marked with different letters for each column are significantly different (P < 0.05).
$^{A, B}$ Significant differences between males and females (P < 0.05).

Regarding the effect of age, it can be seen that age significantly influenced RBC enzyme activity (Fig. 8). It was the lowest in day-old chickens. The enzyme activity increased up to 4 weeks of age and then decreased until the period of higher egg production (Table 12).

Table 12. RBC haemolysate activity (U/g protein) of different age groups (mean ± SD)

Age	RBC haemolysate
1 day	3.30±1.39 [a]
4 weeks	8.91±1.82 [e]
8 weeks	7.80±2.29 [d]
12 weeks	6.49±2.60 [c]
SM	6.80±2.00 [c]
HEP	4.74±2.00 [b]

Means marked with different letters are significantly different ($P < 0.05$).
SM = age at sexual maturation
HEP = age at highest egg production

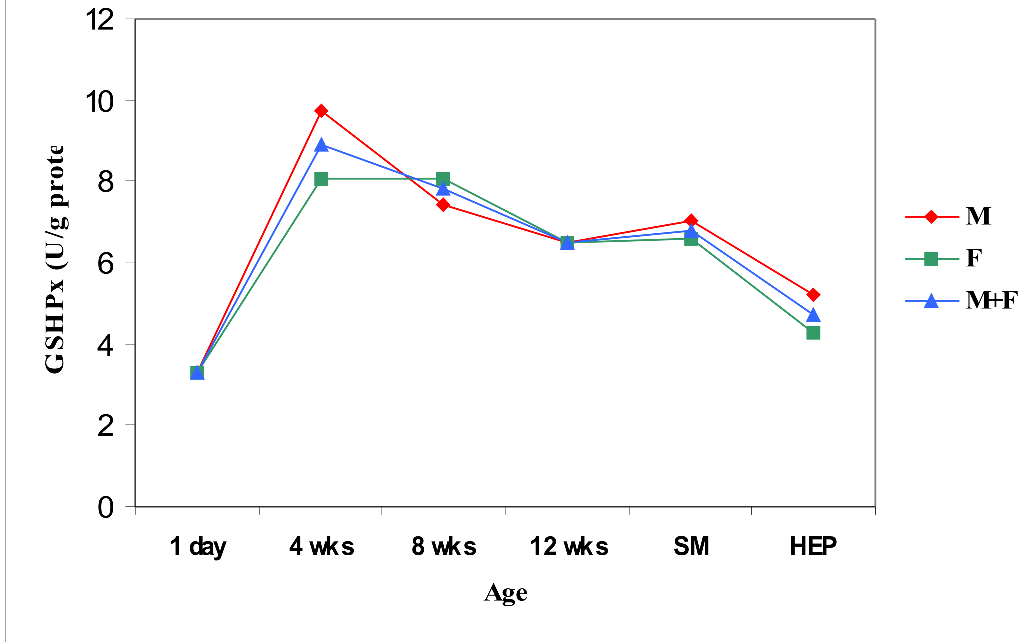


Fig. 8. RBC GSHPx activity of different sexs during development
GSHPx (U/g prote
12
10
8
6
4
2
0
1 day
4 wks
8 wks
12 wks
SM
HEP
Age
M
F
M+F

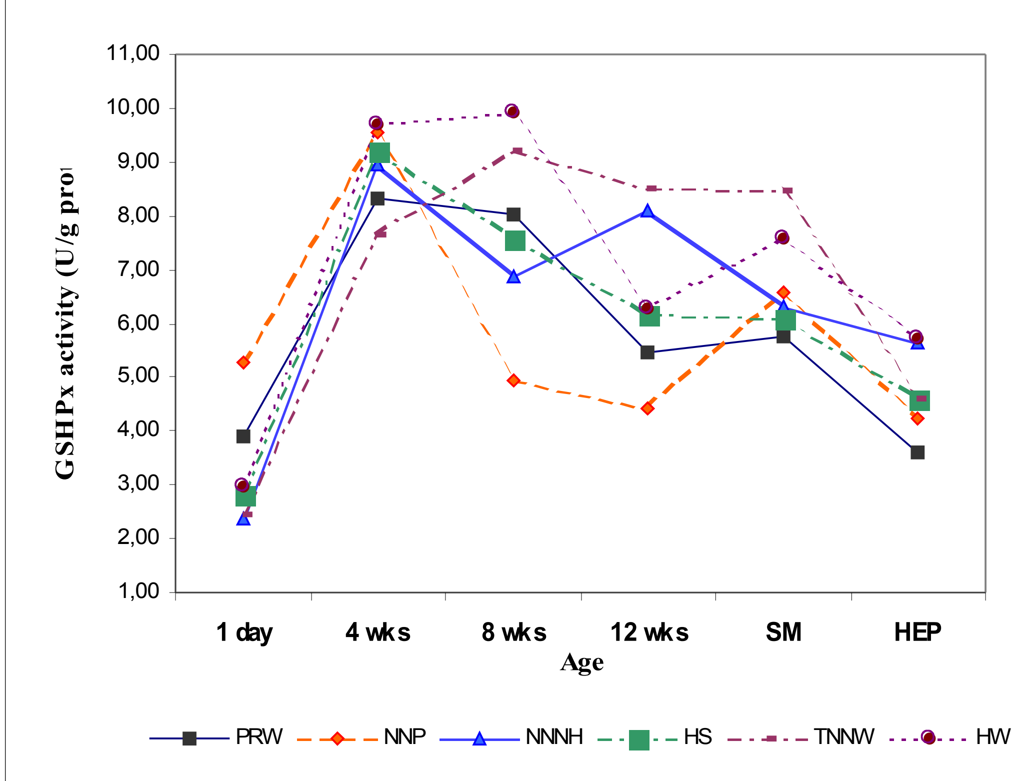


Fig. 9. RBC GSHPx activity of different breeds during development
GSHPx activity (U/g pro
11,00
10,00
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
1 day
4 wks
8 wks
12 wks
SM
HEP
Age
PRW
NNP
NNNH
HS
TNNW
HW

Regarding breed and age interaction, variation of RBC GSHPx activity showed a mixed pattern of increase and decrease depending on breeds (Table 13). There were significant differences in the GSHPx activity of erythrocyte haemolysate among breeds in all age groups, but they were not consequent in different age groups (Fig. 9). At eight weeks of age the highest significant difference between breeds was observed and at the age of sexual maturity these values tended to cequatize (Table 13).

Age and sex interaction was also significant (Table 13). Males showed higher activity at 4, 12 weeks, at sexual maturation and at the age of highest egg production of the layers. Females showed the higher activity at 8 weeks of age (Fig. 8).

Breed, age and sex interaction was statistically significant. The differences between males and females in different age groups have a wide variation among breeds. NNNH and TNNW have significant differences between sexes at three age groups while there were significant differences between sexes at only one age in PRW, NNP and HW (Table 13).

Table 13. RBC haemolysate GSHPx activity (U/g protein) of different breeds, sex and age groups (mean ± SD)

Breed	Day-old	4 weeks	8 weeks	12 weeks	SM	HEP
PRW (M)		10.52±1.5 ^{b}	7.53±1.0	5.71±1.9	6.34±1.5	4.05±1.1
PRW (F)		6.16±1.4 ^{a}	8.49±1.2	5.23±1.2	5.20±1.0	3.18±0.8
PRW (M+F)	3.91±0.2 $^{\mathrm{b}}$	8.34±2.6 $^{\mathrm{ab}}$	8.01±1.2 $^{\mathrm{c}}$	5.47±1.6 $^{\mathrm{ab}}$	5.77±1.4 $^{\mathrm{a}}$	3.61±1.1 $^{\mathrm{a}}$
NNP (M)		10.22±0.8 ^{b}	5.18±1.0	4.89±1.3	6.51±1.8	3.75±1.2
NNP (F)		8.84±0.9 ^{a}	4.70±0.9	3.92±1.0	6.65±1.6	4.74±1.3
NNP (M+F)	5.27±1.4 $^{\mathrm{c}}$	9.53±1.1 $^{\mathrm{bc}}$	4.94±1.0 $^{\mathrm{a}}$	4.41±1.3 $^{\mathrm{a}}$	6.58±1.7 $^{\mathrm{a}}$	4.24±1.3 $^{\mathrm{a}}$
NNNH (M)		9.99±1.5 ^{b}	7.37±2.0	7.27±1.7 ^{a}	6.30±1.6	6.95±2.5 ^{b}
NNNH (F)		7.96±1.0 ^{a}	6.41±1.0	8.90±2.6 ^{b}	6.31±1.8	4.36±2.7 ^{a}
NNNH (M+F)	2.37±0.7 $^{\mathrm{a}}$	8.97±1.6 $^{\mathrm{bc}}$	6.89±1.6 $^{\mathrm{b}}$	8.08±2.3 $^{\mathrm{c}}$	6.30±1.7 $^{\mathrm{a}}$	5.65±2.9 $^{\mathrm{b}}$
HS (M)		9.79±1.2 ^{b}	7.06±1.1	7.82±2.5 ^{b}	5.76±1.0	4.56±0.5
HS (F)		8.63±0.6 ^{a}	8.07±1.3	4.55±1.2 ^{a}	6.44±2.2	4.62±0.8
HS (M+F)	2.82±1.1 $^{\mathrm{a}}$	9.21±1.1 $^{\mathrm{bc}}$	7.56±1.3 $^{\mathrm{bc}}$	6.18±2.6 $^{\mathrm{b}}$	6.10±1.7 $^{\mathrm{a}}$	4.59±0.7 $^{\mathrm{b}}$
TNNW (M)		8.22±2.1	8.56±2.1 ^{a}	6.83±1.7 ^{a}	9.11±2.0	6.39±2.6 ^{b}
TNNW (F)		7.12±2.1	9.85±1.3 ^{b}	10.19±2.0 ^{b}	7.81±2.1	2.86±1.3 ^{a}
TNNW (M+F)	2.44±0.7 $^{\mathrm{a}}$	7.67±2.1 $^{\mathrm{a}}$	9.21±1.8 $^{\mathrm{d}}$	8.51±2.9 $^{\mathrm{c}}$	8.46±2.4 $^{\mathrm{b}}$	4.62±2.7 $^{\mathrm{a}}$
HW (M)		9.63±1.2	8,83±2.5 ^{a}	6.55±1.7	8.15±2.1	5.61±1.2
HW (F)		9.77±2.0	11.02±1.9 ^{b}	6.03±2.5	7.03±1.2	5.80±1.3
HW (M+F)	2.99±0.6 $^{\mathrm{a}}$	9.70±1.6 $^{\mathrm{c}}$	9.93±2.5 $^{\mathrm{c}}$	6.29±2.1 $^{\mathrm{b}}$	7.59±1.7 $^{\mathrm{b}}$	5.71±1.3 $^{\mathrm{b}}$
SEX effect						
Male		9.73±1.6 ^{B}	7.42±2.1 ^{A}	6.51±2.0	7.03±2.2 ^{B}	5.22±2.0 ^{B}
Female		8.08±1.8 ^{A}	8.09±2.5 ^{B}	6.47±3.1	6.57±1.8 ^{A}	4.26±1.8 ^{A}

$^{\mathrm{a,\,b}}$ Means marked with different letters within each column are significantly different ($P < 0.05$).
^{a,b} Significant differences within the breed between males and females ($P < 0.05$).
$^{A,\,B}$ Significant differences within the age between males and females ($P < 0.05$).
SM = age at sexual maturation
HEP = age at highest egg production

5. 2. 3. Phenotypic variation of GSHPx activity of blood plasma

Phenotypic variations of GSHPx activity of blood plasma in different breeds are summarised in Table 14. Concerning the breed effect, PRW breed had the highest enzyme activity and NNP had the lowest (Fig. 7). The same tendency was found for females. While HS showed the highest blood plasma enzyme activity and NNP had the lowest for males.

Breed and sex interaction was significant. Males had higher blood plasma enzyme activity in NNP, HS, TNNW and HW breeds and females showed higher enzyme activity in PRW and NNNH breeds (Table 14).

Regardless of age and breed effects, sex had a significant influence on blood plasma GSHPx activity. Males had higher activity than females (Table 14).

Table 14. Blood plasma GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	Blood plasma GSHPx activity		
	Male	**Female**	**M+F**
PRW	6.95 ± 1.9^{b}	7.59 ± 2.8^{c}	7.27 ± 2.4^{d}
NNP	6.27 ± 1.7^{a}	6.01 ± 1.5^{a}	6.14 ± 1.6^{a}
NNNH	6.33 ± 2.1^{a}	6.56 ± 2.0^{ab}	6.45 ± 2.0^{b}
HS	7.52 ± 2.1^{c}	6.34 ± 2.5^{a}	6.93 ± 2.4^{c}
TNNW	7.12 ± 2.0^{bc}	6.87 ± 2.2^{b}	6.99 ± 2.1^{cd}
HW	6.70 ± 2.4^{b}	6.25 ± 2.4^{a}	6.47 ± 2.4^{b}
Sex effect	6.81 ± 2.1^{B}	6.60 ± 2.3^{A}	

$^{a, b}$ Means marked with different letters for each column are significantly different ($P < 0.05$).
$^{A, B}$ Significant differences between males and females ($P < 0.05$).

It is clearly obvious that age also significantly influenced blood plasma enzyme activity (Table 15). It decreased from hatching until the age of sexual maturation and there was a moderate increase at the age of highest egg production of the layers (Fig. 10).

Table 15. Blood plasma activity (U/g protein) of different age groups (mean ± SD)

Age	Blood plasma
1 day	9.18±2.02 [e]
4 weeks	8.83±2.40 [d]
8 weeks	7.68±2.00 [c]
12 weeks	5.60±1.70 [ab]
SM	5.42±1.20 [a]
HEP	5.89±1.30 [b]

Means marked with different letters are significantly different ($P < 0.05$).
SM = age at sexual maturation
HEP = age at highest egg production

The results also showed that there are significant differences in blood plasma enzyme activity between breeds in all age groups. The differences were not consequent for different age groups (Fig. 11). The age at sexual maturation was associated with the widest significant differences among breeds and at the age of highest egg production it was the narrowest (Table 16). Variation of blood plasma GSHPx activity showed mixed pattern of increase and decrease as an effect of ageing, but it was breed dependent (Table 16).

Age and sex interaction was also significant (Table 16). Males showed significantly higher activity at 4 weeks, at the age of sexual maturity and of higher egg production while females showed the higher activity at 8 and 12 weeks of age (Fig. 10).

Breed, age and sex interaction was also found. The differences between males and females in each age group showed a wide variation among breeds (Table 16).

Table 16. Blood plasma GSHPx activity (U/g protein) of different breeds, sex during development (mean ± SD)

Breed	Day-old	4 weeks	8 weeks	12 weeks	SM	HEP
PRW (M)		9.12±0.8 ^{a}	5.83±1.1	5.53±1.3 ^{a}	6.78±0.7	6.72±1.0
PRW (F)		13.14±1.6 ^{b}	6.74±1.2	7.48±1.4 ^{b}	6.66±1.3	5.23±2.0
PRW (M+F)	10.37±1.7 $^{\mathrm{c}}$	11.13±2.4 $^{\mathrm{c}}$	6.28±1.2 $^{\mathrm{a}}$	6.50±1.7 $^{\mathrm{b}}$	6.72±1.0 $^{\mathrm{d}}$	5.97±1.7 $^{\mathrm{ab}}$
NNP (M)		8.40±1.1 ^{b}	6.20±1.7	4.60±1.1	5.90±0.8 ^{b}	6.01±0.8
NNP (F)		6.47±1.1 ^{a}	6.60±1.1	5.22±1.3	4.57±0.9 ^{a}	6.44±1.2
NNP (M+F)	8.92±0.8 $^{\mathrm{b}}$	7.43±1.5 $^{\mathrm{a}}$	6.40±1.4 $^{\mathrm{a}}$	4.91±1.2 $^{\mathrm{a}}$	5.24±1.1 $^{\mathrm{b}}$	6.23±1.0 $^{\mathrm{b}}$
NNNH (M)		6.73±0.5 ^{a}	7.13±1.2	5.43±1.1 ^{a}	4.56±0.4	6.01±1.0
NNNH (F)		7.45±1.0 ^{b}	6.75±1.3	7.00±1.1 ^{b}	3.97±0.5	6.55±1.1
NNNH (M+F)	11.40±2.3 $^{\mathrm{c}}$	7.09±0.9 $^{\mathrm{a}}$	6.94±1.3 $^{\mathrm{a}}$	6.22±1.4 $^{\mathrm{b}}$	4.26±0.6 $^{\mathrm{a}}$	6.28±1.1 $^{\mathrm{b}}$
HS (M)		10.92±1.8 ^{b}	8.92±1.5	6.23±1.1 ^{b}	6.39±0.9 ^{b}	6.41±1.2 ^{b}
HS (F)		8.09±1.3 ^{a}	9.76±1.3	3.49±0.8 ^{a}	5.66±1.2 ^{a}	4.79±0.5 ^{a}
HS (M+F)	7.48±1.2 $^{\mathrm{a}}$	9.50±2.1 $^{\mathrm{b}}$	9.34±1.5 $^{\mathrm{c}}$	4.86±1.7 $^{\mathrm{a}}$	6.03±1.1 $^{\mathrm{c}}$	5.60±1.2 $^{\mathrm{ab}}$
TNNW (M)		7.69±0.8 ^{a}	9.39±2.1	6.30±1.1	5.48±0.8 ^{b}	6.20±1.0
TNNW (F)		8.50±1.9 ^{b}	8.28±2.2	6.97±1.3	4.59±0.7 ^{a}	5.74±2.0
TNNW (M+F)	9.31±1.4 $^{\mathrm{b}}$	8.09±1.5 $^{\mathrm{a}}$	8.83±2.2 $^{\mathrm{bc}}$	6.63±1.2 $^{\mathrm{b}}$	5.03±0.9 $^{\mathrm{b}}$	5.97±1.6 $^{\mathrm{ab}}$
HW (M)		11.15±3.2 ^{b}	7.11±1.0 ^{a}	5.39±1.4 ^{b}	5.22±0.8	5.74±0.5 ^{b}
HW (F)		8.32±0.6 ^{a}	9.50±1.4 ^{b}	3.51±0.7 ^{a}	5.28±0.1	4.89±0.8 ^{a}
HW (M+F)	7.61±1.1 $^{\mathrm{a}}$	9.74±2.7 $^{\mathrm{b}}$	8.30±1.7 $^{\mathrm{b}}$	4.45±1.4 $^{\mathrm{a}}$	5.25±0.9 $^{\mathrm{b}}$	5.32±0.8 $^{\mathrm{a}}$
Sex effect						
Male		9.00±2.3 ^{B}	7.43±2.0 ^{A}	5.58±1.3	5.72±1.0 ^{B}	6.18±1.0 ^{B}
Female		8.66±2.5 ^{A}	7.93±2.0 ^{B}	5.61±2.0	5.12±1.3 ^{A}	5.61±1.5 ^{A}

$^{\mathrm{a,b}}$ Means marked with different letters within each column are significantly different ($P < 0.05$).
^{a,b} Significant differences within the breed between males and females ($P < 0.05$).
$^{A,\,B}$ Significant differences within the age between males and females ($P < 0.05$).
SM = age at sexual maturation
HEP = age at highest egg production

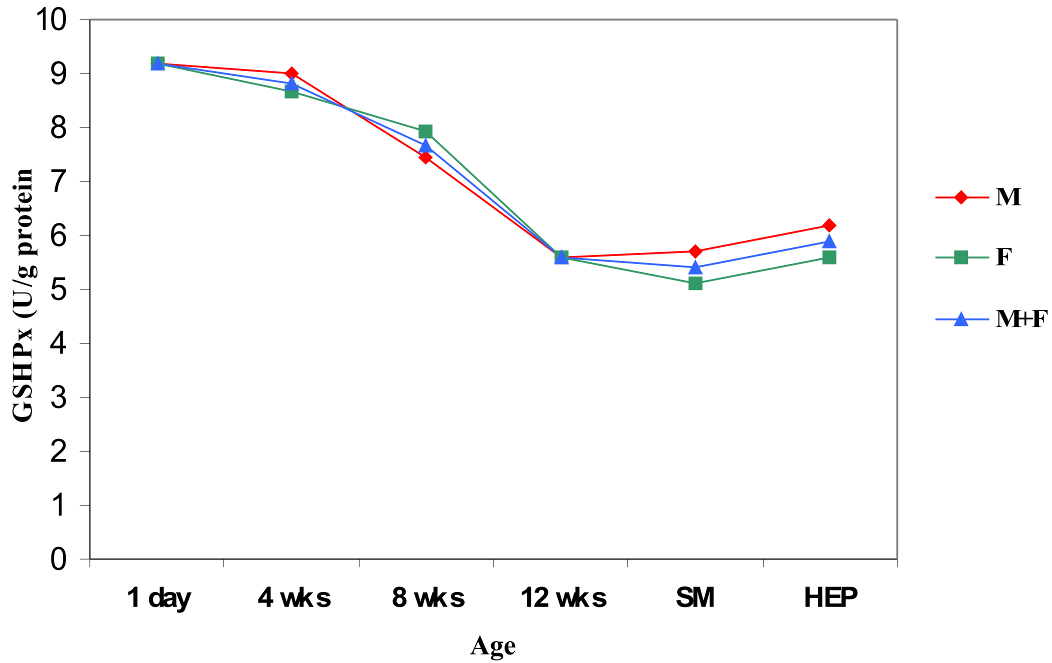


Fig. 10. Blood plasma GSHPx activity of different sexs during development
GSHPx (U/g protein
10
9
8
7
6
5
4
3
2
1
0
1 day
4 wks
8 wks
12 wks
SM
HEP
Age
M
F
M+F

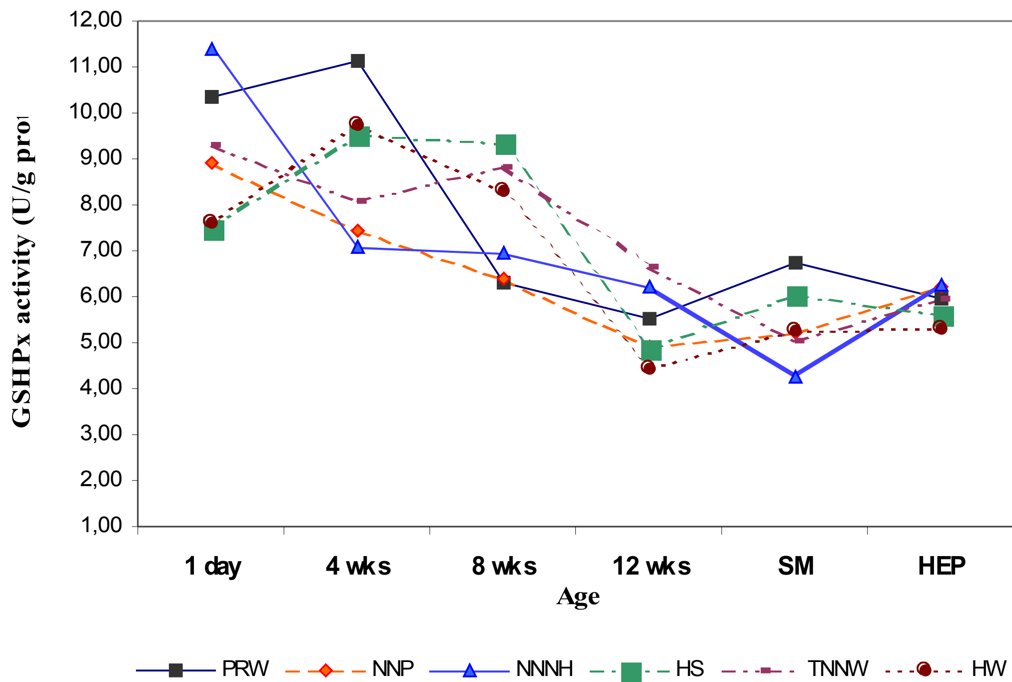


Fig. 11. Blood Plasma GSHPx activity of different breeds during development
GSHPx activity (U/g pro
12,00
11,00
10,00
9,00
8,00
7,00
6,00
5,00
4,00
3,00
2,00
1,00
1 day
4 wks
8 wks
12 wks
SM
HEP
Age
PRW
NNP
NNNH
HS
TNNW
HW

5. 2. 4. Phenotypic variation of body weight

Average body weight measurements are given in Table 17. Significant differences were found among breeds at all age groups, PRW had the highest BW at 4, 12 weeks of age, SM and at the age of highest egg production and NNP had the highest at 8 weeks of age. HW had the lowest BW at 4 and 8 weeks and TNNW had the lowest BW at 12 weeks, SM and at the age of highest egg production. Males had significant higher BW than females at the age of SM and higher egg production.

Table 17. Body weight (kg) of different breeds, sex and age groups (mean ± SD)

Breed	4 weeks	8 weeks	12 weeks	SM	HEP
PRW (M)	0.34±0,01	0.79±0.13	1.43±0.21	3.07±0.27 ^{b}	3.39±0.18 ^{b}
PRW (F)	0.31±0.01	0.80±0.12	1.45±0.20	2.45±0.24 ^{a}	2.34±0.23 ^{a}
PRW (M+F)	0.33±0.01 $^{\mathrm{c}}$	0.79±0.12 $^{\mathrm{c}}$	1.44±0.20 $^{\mathrm{d}}$	2.76±0.41 $^{\mathrm{b}}$	2.86±0.57 $^{\mathrm{c}}$
NNP (M)	0.31±0.01	0.90±0.01 ^{b}	1.44±0.21 ^{b}	2.96±0.36 ^{b}	3.31±0.37 ^{b}
NNP (F)	0.34±0.01	0.79±0.01 ^{a}	1.19±0.20 ^{a}	2.15±0.28 ^{a}	2.30±0.33 ^{a}
NNP (M+F)	0.32±0.01 $^{\mathrm{c}}$	0.84±0.01 $^{\mathrm{d}}$	1.32±0.24 $^{\mathrm{c}}$	2.55±0.52 $^{\mathrm{b}}$	2.80±0.61 $^{\mathrm{c}}$
NNNH (M)	0.32±0.01	0.83±0.12 ^{b}	1.18±0.19	3.18±0.20 ^{b}	3.32±0.27 ^{b}
NNNH (F)	0.29±0.01	0.72±0.13 ^{a}	1.30±0.13	2.18±0.29 ^{a}	2.09±0.27 ^{a}
NNNH (M+F)	0.31±0.01 $^{\mathrm{c}}$	0.78±0.13 $^{\mathrm{c}}$	1.24±0.17 $^{\mathrm{c}}$	2.68±0.56 $^{\mathrm{b}}$	2.71±0.68 $^{\mathrm{c}}$
HS (M)	0.21±0.01	0.56±0.01 ^{a}	1.01±0.17	3.04±0.15 ^{b}	2.51±0.18 ^{b}
HS (F)	0.23±0.01	0.72±0.01 ^{b}	1.04±0.15	2.00±0.24 ^{a}	2.00±0.22 ^{a}
HS (M+F)	0.22±0.01 $^{\mathrm{a}}$	0.64±0.11 $^{\mathrm{b}}$	1.02±0.16 $^{\mathrm{a}}$	2.52±0.57 $^{\mathrm{b}}$	2.25±0.33 $^{\mathrm{b}}$
TNNW (M)	0.32±0.01 ^{b}	0.50±0.01	0.85±0.14	2.18±0.29 ^{b}	2.05±0.23 ^{b}
TNNW (F)	0.20±0.01 ^{a}	0.56±0.01	0.89±0.11	1.48±0.18 ^{a}	1.59±0.22 ^{a}
TNNW (M+F)	0.26±0.01 $^{\mathrm{b}}$	0.53±0.01 $^{\mathrm{a}}$	0.87±0.13 $^{\mathrm{a}}$	1.83±0.43 $^{\mathrm{a}}$	1.82±0.32 $^{\mathrm{a}}$
HW (M)	0.23±0.01	0.57±0.01	1.05±0.12	2.23±0.24 ^{b}	2.28±0.17 ^{b}
HW (F)	0.20±0.01	0.60±0.01	0.92±0.13	1.77±0.25 ^{a}	1.92±0.24 ^{a}
HW (M+F)	0.22±0.01 $^{\mathrm{a}}$	0.58±0.01 $^{\mathrm{a}}$	0.99±0.14 $^{\mathrm{b}}$	2.00±0.33 $^{\mathrm{a}}$	2.10±0.27 $^{\mathrm{b}}$
Sex effect					
Male	0.29±0.01	0.69±0.18	1.16±0.28	2.78±0.48 ^{B}	2.81±0.60 ^{B}
Female	0.26±0.01	0.70±0.13	1.13±0.25	2.00±0.40 ^{A}	2.04±0.35 ^{A}

$^{\mathrm{a,\,b}}$ Means marked with different letters within each column are significantly different (P < 0.05).
^{a,b} Significant differences within the breed between males and females (P < 0.05).
$^{A,\,B}$ Significant differences within the age between males and females (P < 0.05).
SM = age at sexual maturation
HEP = age at highest egg production

5. 2. 5. Phenotypic correlation between body weight and liver, RBC and blood plasma GSHPx activity

Negative correlations were found between GSHPx activity in newly hatched chicks' liver and body weight of all age groups (Table 18).

The same negative correlation was found between GSHPx activity of RBC haemolysate and body weight of all age groups (Table 18). Regardless of age, highly significant negative correlation was found between GSHPx activity of RBC and body weight (Table 18). Also negative correlation was found between GSHPx activity in blood plasma and body weight at all age groups except at four weeks (Table 18). Regardless of age, highly significant negative correlation was found between GSHPx activity in blood plasma and body weight (Table 19).

5. 2. 6. Phenotypic correlation between egg production and liver, RBC and blood plasma GSHPx activity

Non significant negative correlations were found between GSHPx activity in liver and RBC with egg production and significant positive correlation between GSHPx activity in blood plasma and egg production (0,83; $P \leq 0.05$) (Table 18).

5. 2. 7. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity

Significant negative correlation was found between liver enzyme activity at day old with blood plasma GSHPx activity of 12 weeks (-0.50; $P \leq 0.05$) and at the highest egg production period (0.55; $P \leq 0.05$). While, positive correlation was found with RBC at 4 weeks (0.40; $P \leq 0.05$) and 8 weeks (0.50; $P \leq 0.05$).

Significant negative correlation was found between blood plasma enzyme activity and RBC GSHPx activity at day-old (0.20; $P \leq 0.05$), 4 weeks (0.20; $P \leq 0.05$). While, significant positive correlation were found at 8 weeks (0.30; $P \leq 0.01$) and at 12 weeks (0.30; $P \leq 0.01$). Regardless of age effect, significant positive correlation was found between blood plasma and RBC GSHPx activity (0.20; $P \leq 0.01$).

Table 18. Phenotypic correlations between GSHPx activity in RBC haemolysate, blood plasma and liver homogenate 10,000 g supernatant (U/g protein) and body weight (BW) and egg production

	BW 4 wks	BW 8 wks	BW 12 wks	BW SM	BW HEP	Egg production
			RBC haemolysate			
1 day	0.14	0.60	0.60	0.26	0.54	-0.49
4 wks	-0.37	0.20	0.20	-0.09	0.03	-0.14
8 wks	-0.60	-0.77	-0.77	-0.54	-0.60	-0.77
12 wks	-0.43	-0.83	-0.83*	-0.54	-0.77	0.09
SM+	-0.77	-0.60	-0.60	-0.83*	-0.77	-0.26
HEP++	-0.60	-0.54	-0.54	-0.43	-0.60	0.09
Overall mean	**-0.66**	**-0.94****	**-0.94****	**-0.71**	**-0.89***	**-0.20**
			Blood plasma			
1 day	0.66	0.26	0.26	0.54	0.37	0.20
4 wks	-0.10	-0.14	-0.14	0.03	0.09	-0.77
8 wks	-0.71	-0.77	-0.77	-0.77	-0.83*	0.20
12 wks	0.43	-0.03	-0.03	0.14	0.09	0.09
SM+	0.14	0.20	0.20	0.26	0.37	-0.49
HEP++	0.77	0.66	0.66	0.66	0.60	0.60
Overall mean	**0.20**	**-0.14**	**-0.14**	**-0.03**	**-0.20**	**0.83***
			Liver homogenate 10,000 g supernatant			
1 day	-0.49	-0.60	-0.60	-0.31	-0.54	-0.03

* Correlation is significant at P< 0.05 level.
** Correlation is significant at P< 0.01 level.
SM = age at sexual maturation
HEP = age at highest egg production

Table 19. Phenotypic correlation between GSHPx activity in RBC and blood plasma and body weight

Body weight at	RBC	Blood plasma
4 weeks	0.10	0.25**
8 weeks	-0.40**	-0.33**
12 weeks	-0.25**	0.06
Sexual maturation	0.24**	0.34**
Highest egg production	0.01	0.17*
Overall mean	**-0.48****	**-0.46****

* Correlation is significant at P< 0.05 level.
** Correlation is significant at P< 0.01 level.

5. 3. Experiment 3: Phenotypic variation in the GSHPx activity in two chicken breeds and their crosses of different ages from 1-day to the age of sexual maturation

In a preliminary experiment, some breeds of the same age were tested for GSHPx activity and the breed was chosen which showed the highest enzyme activity (New Hampshire) and the lowest (Transylvanian Naked Neck Black) and made a cross (New Hampshire sires crossed Transylvanian Naked Neck Black dames) and its reciprocal one (New Hampshire dames crossed Transylvanian Naked Neck Black sires) to study the phenotypic variation in the activity of GSHPx in liver, blood plasma and RBC in these two breeds and their crosses and their correlation with some production traits. We have studied the effect of heterosis and reciprocal effect on the enzyme activity and body weight.

5. 3. 1. Phenotypic variation of liver GSHPx activity

Phenotypic variations of GSHPx activity of liver homogenate in different breeds are summarised in Table 20. Significant differences in the GSHPx activity of liver were found between the two breeds and crosses, NH had higher activity than TNNB. The N x T cross had higher activity than the reciprocal one and the two crosses had lower activity than their parents (Fig. 12).

Table 20. Liver homogenate 10,000 g supernatant, GSHPx activity (U/g protein) of different breeds (mean ± SD)

Breed	Liver GSHPx activity
NH	1.83±0.13 [d]
TNNB	1.68±0.05 [c]
N x T	1.54±0.25 [b]
T x N	1.27±0.20 [a]

Means marked with different letters are significantly different ($P < 0.05$).

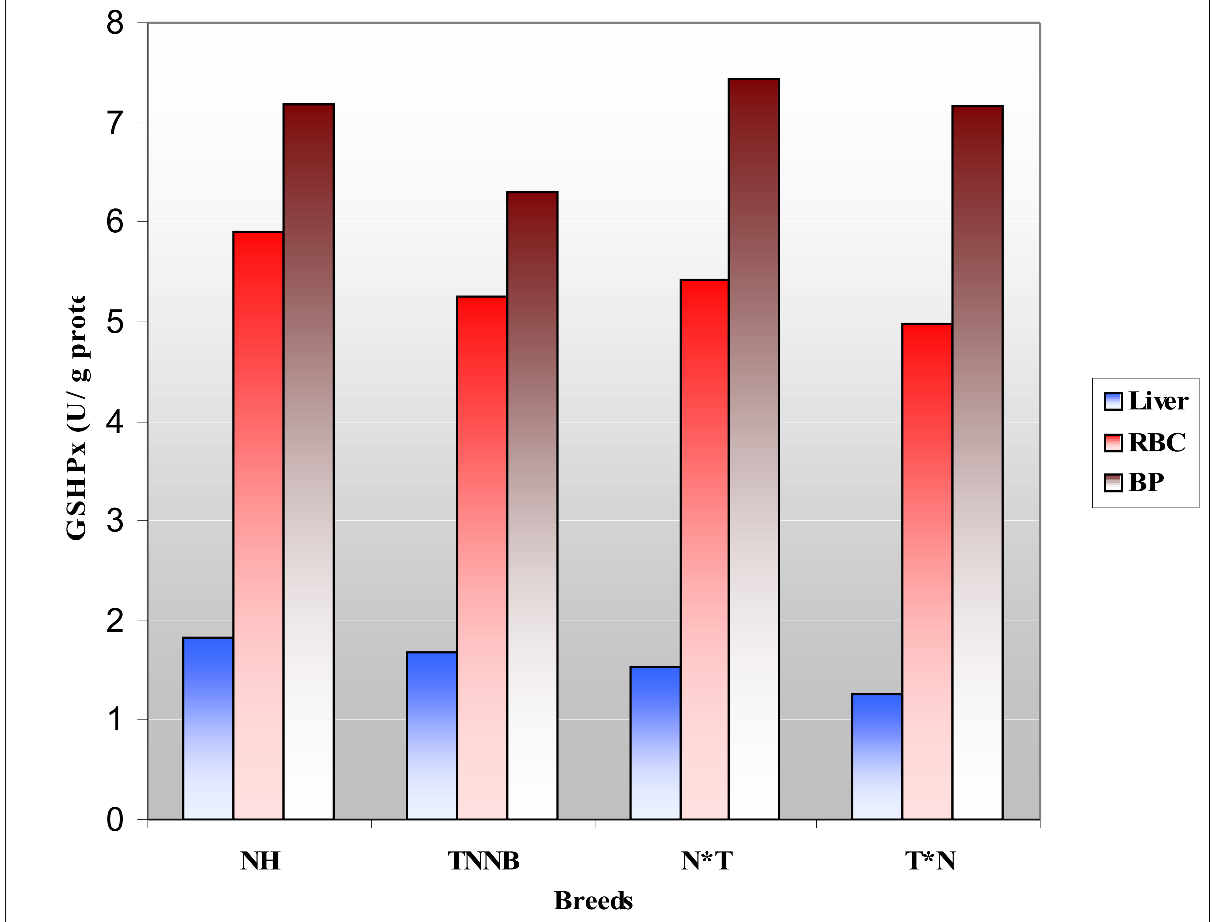


Fig. 12. Liver, RBC and blood plasma GSHPx of different breeds and crosses
GSHPx (U/ g prote
8
7
6
5
4
3
2
1
0
NH
TNNB
N*T
T*N
Breeds
Liver
RBC
BP

5. 3. 2. Phenotypic variation of GSHPx activity of RBC haemolysate

Phenotypic variations of GSHPx activity of RBC in different breeds are given in Table 21. Regarding the breed effect, as found in liver, NH had higher activity than TNNB breed. The N x T cross had higher activity than the reciprocal one. (Fig. 12). The same tendency was found for males and females (Table 21).

Sex had also significant influence on erythrocyte haemolysate GSHPx activity, males having higher enzyme activity than females (Table 21).

Table 21. Red blood cell GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	RBC GSHPx activity		
	Male	**Female**	**M+F**
NH	6.25±2.04 [c]	5.56±1.87 [c]	5.89±1.98 [c]
TNNB	5.20±1.88 [b]	5.30±1.51 [b]	5.25±1.69 [ab]
N x T	5.58±1.83 [bc]	5.26±1.65 [b]	5.42±1.74 [b]
T x N	4.96±1.87 [a]	4.99±2.00 [a]	4.97±1.94 [a]
Sex effect	5.49±1.95 [B]	5.28±1.77 [A]	

[a, b] Means marked with different letters for each column are significantly different ($P < 0.05$).
[A, B] Significant differences between males and females ($P < 0.05$).

Regarding breed and sex interaction, male had higher enzyme activity than female in NH and N x T crosses as shown in (Table 21).

Regarding the effect of age, it can be seen that age significantly influenced the RBC enzyme activity (Fig. 13), it was the lowest at four weeks and highest at eight weeks of age (Table 22).

Table 22. Red blood cell GSHPx activity (U/g protein) of different age groups (mean ± SD)

Age	RBC GSHPx activity
1 day	5.25±2.16 [c]
4 weeks	3.77±1.02 [a]
8 weeks	6.62±1.52 [e]
12 weeks	4.63±1.64 [b]
SM	5.75±1.67 [d]

Means marked with different letters are significantly different ($P < 0.05$).
SM = age at sexual maturation

Breed and age interaction showed that, there are significant differences in the GSHPx activity of erythrocyte haemolysate between the two breeds and crosses in all age groups (Table 23). Those differences were not consequent in different age groups (Fig. 14). NH had higher activity than TNNB at one day, 12 weeks of age and the age of sexual maturity. The N x T cross had higher activity than the reciprocal cross at day old, 4, 12 weeks of age and the age of sexual maturity. The two crosses had lower enzyme activity than the two parental breeds at 12 weeks of age and the age of sexual maturity (Table 23). Breed, age and sex interaction was significant. The differences between males and females in each age had a wide variation between the two breeds and the crosses (Table 23).

Table 23. RBC haemolysate GSHPx activity (U/g protein) of different breeds, sex and

age groups (mean ± SD)

Breed	Day-old	4 weeks	8 weeks	12 weeks	SM
NH (M)		3.18±0.55	6.82±1.53	6.17±1.75	7.78±1.53 ^{b}
NH (F)		3.27±0.93	6.01±0.70	5.89±1.97	6.30±2.23 ^{a}
NH (M+F)	4.61±0.74 ^{a}	3.23±0.75 ^{a}	6.42±1.24 ^{a}	6.02±1.84 ^{b}	6.99±2.04 ^{b}
TNNB (M)		2.95±0.20 ^{a}	6.78±1.54	4.43±0.81	6.07±1.67
TNNB (F)		4.52±1.16 ^{b}	6.52±1.68	4.45±0.84	5.46±1.10
TNNB (M+F)	4.13±1.68 ^{a}	3.79±1.17 ^{a}	6.65±1.59 ^{a}	4.44±0.81 ^{a}	5.76±1.42 ^{a}
N x T (M)		4.64±0.71	6.74±1.61	4.21±1.57	5.45±0.89
N x T (F)		4.80±1.11	5.93±1.49	4.27±1.53	5.04±0.89
N x T (M+F)	7.77±2.09 ^{b}	4.72±0.92 ^{b}	6.34±1.58 ^{a}	4.24±1.52 ^{a}	5.24±0.90 ^{a}
T x N (M)		3.21±0.25	6.92±1.46	3.69±1.26	5.26±1.59
T x N (F)		3.43±0.33	7.24±1.79	3.99±1.48	4.80±1.27
T x N (M+F)	4.49±1.69 ^{a}	3.32±0.31 ^{a}	7.08±1.6 b	3.84±1.36 ^{a}	5.02±1.43 ^{a}
Sex effect					
Male		3.49±0.82 ^{A}	6.82±1.50 ^{B}	4.60±1.63	6.11±1.72 ^{B}
Female		4.00±1.12 ^{B}	6.42±1.53 ^{A}	4.66±1.66	5.40±1.55 ^{A}

$^{a, b}$ Means marked with different letters within each column are significantly different (P < 0.05).

$^{a,.b}$ Significant differences within the breed between males and females (P < 0.05).

$^{A, B}$ Significant differences within the age between males and females (P < 0.05).

SM = age at sexual maturation

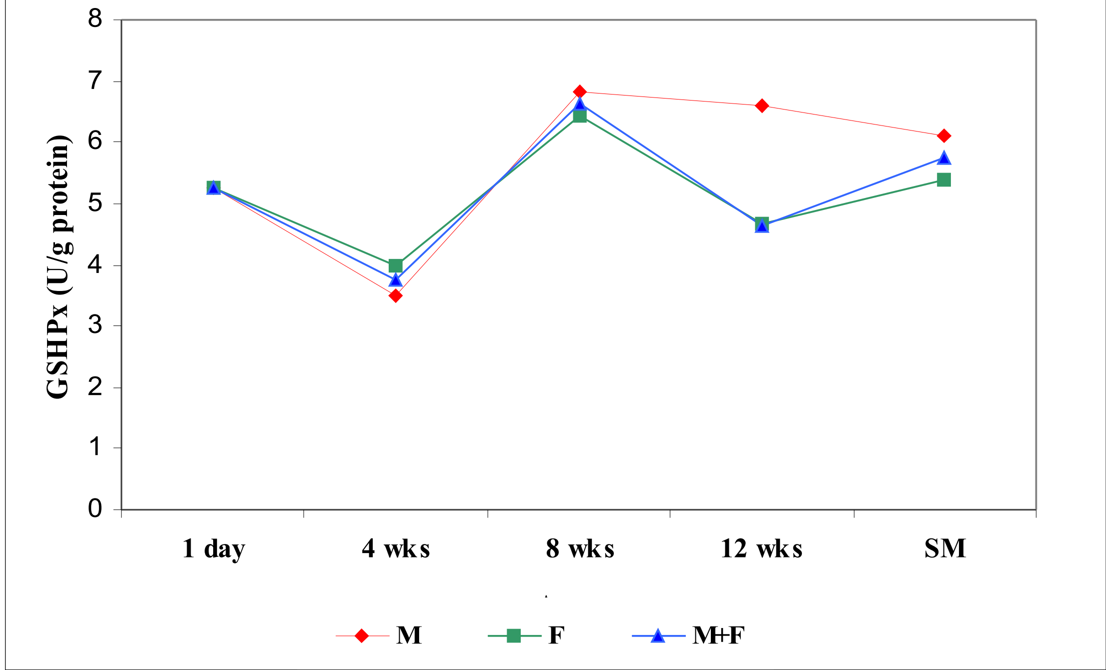


Fig. 13. RBC GSHPx activity of different sex during development
GSHPx (U/g protein)
8
7
6
5
4
3
2
1
0
1 day
4 wks
8 wks
12 wks
SM
M
F
M+F

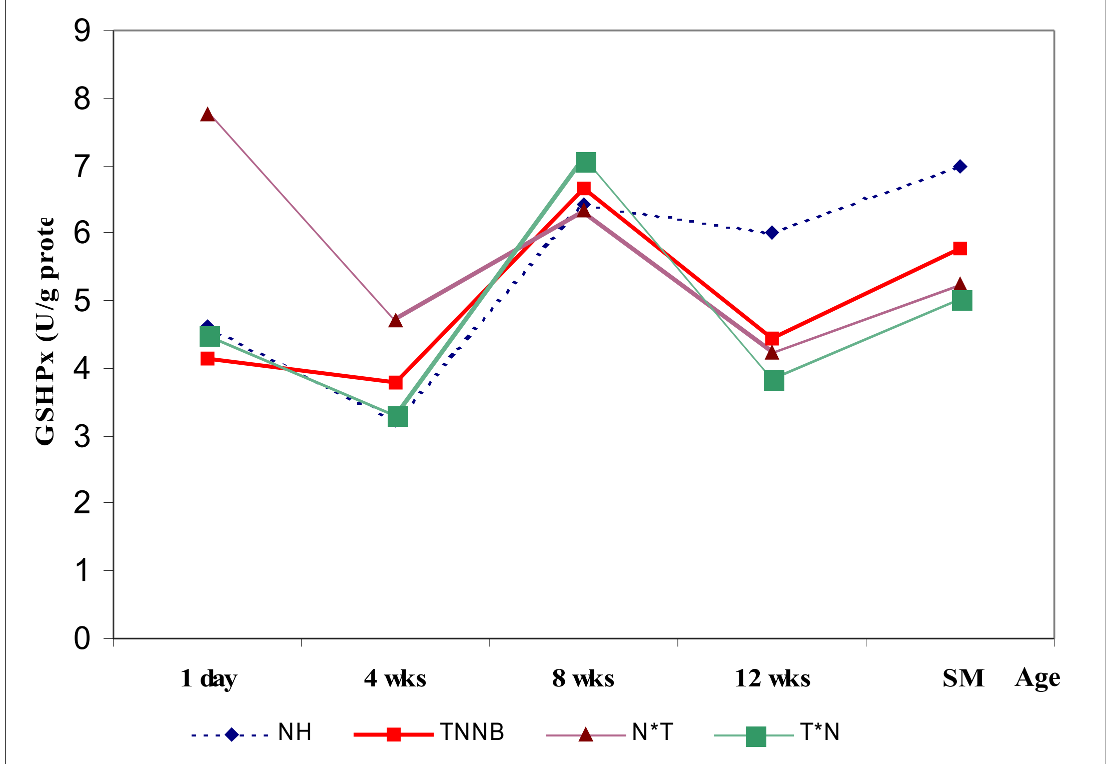


Fig. 14. RBC GSHPx activity of different breeds during development
GSHPx (U/g prote
9
8
7
6
5
4
3
2
1
0
1 day
4 wks
8 wks
12 wks
SM
Age
NH
TNNB
N*T
T*N

5. 3. 3. Phenotypic variation of GSHPx activity of blood plasma

Significant differences in the enzyme activity of blood plasma were found between the two breeds and crosses in all age groups. Regarding the breed effect, as found in liver and RBC, NH had higher activity than TNNB. The N x T cross had higher activity than the reciprocal one (Fig. 12). The same tendency was found in females but T x N cross had higher activity than N x T for males (Table 24).

Sex had considerable influence on blood plasma GSHPx activity, males had significantly higher enzyme activity than females (Table 24).

Concerning breed and sex interaction, male had higher enzyme activity than female in all breeds except N x T as shown in Table 24.

Table 24. Blood plasma GSHPx activity (U/g protein) of different breeds and sex (mean ± SD)

Breed	Blood plasma GSHPx activity		
	Male	Female	M+F
NH	7.43±2.35 ^{b}	6.97±2.49 ^{b}	7.19±2.43 ^{b}
TNNB	6.50±3.18 ^{a}	6.09±2.87 ^{a}	6.29±3.02 ^{a}
N x T	6.88±2.63 ^{a}	7.98±2.89 ^{c}	7.44±2.81 ^{b}
T x N	7.63±2.72 ^{b}	6.69±2.44 ^{b}	7.15±2.61 ^{b}
Sex effect	7.11±2.76 ^{B}	6.93±2.75 ^{A}	

$^{a, b}$ Means marked with different letters for each column are significantly different (P < 0.05).
$^{A, B}$ Significant differences between males and females (P < 0.05).

Regarding the age effect, it can be seen that age influenced significantly blood plasma enzyme activity (Table 25). Enzyme activity decreased with age from day old until 12 weeks and started to increase at the age of sexual maturity (Fig. 15).

Table 25. Blood plasma GSHPx activity (U/g protein) of different age groups (mean ± SD)

Age	Blood plasma GSHPx activity
1 day	13.34±2.13 ^{e}
4 weeks	8.99±1.43 ^{d}
8 weeks	6.63±1.52 ^{c}
12 weeks	5.36±1.68 ^{a}
SM	5.99±1.49 ^{b}

Means marked with different letters are significantly different ($P < 0.05$).
SM = age at sexual maturation

Regarding breed and age interaction, NH has higher activity than TNNB at 4, 8, 12 weeks of age and the age of sexual maturity. The N x T cross had higher activity than the reciprocal one at day old, 12 weeks of age and at age of sexual maturity (Table 26).

Breed, age and sex interaction was significant. The differences between males and females at each age have a wide variation between the two lines and two crosses (Table 26).

Table 26. Blood plasma GSHPx activity (U/g protein) of different breeds, sex and age groups (mean ± SD)

Breed	Day-old	4 weeks	8 weeks	12 weeks	SM
NH (M)		10.75±0.67	7.10±1.11	5.28±1.24	6.95±1.44
NH (F)		10.77±0.76	6.43±0.89	5.05±1.29	6.14±1.42
NH (M+F)	11.23±1.74 [a]	10.76±0.69 [b]	6.76±1.05 [b]	5.16±1.25 [a]	6.52±1.46 [b]
TNNB (M)		8.22±0.65	5.34±0.92	4.39±0.86	6.03±1.27 [b]
TNNB (F)		8.23±1.24	4.64±1.36	4.93±1.64	5.12±1.05 *[a]*
TNNB (M+F)	14.37±2.06 [b]	8.23±0.98 [a]	4.99±1.20 [a]	4.66±1.32 [a]	5.58±1.24 [a]
N x T (M)		7.93±1.17 *[a]*	6.48±0.82 *[a]*	5.10±1.37 *[a]*	6.36±1.67 *[b]*
N x T (F)		8.72±1.33 *[b]*	8.15±1.01 *[b]*	7.33±2.37 *[b]*	5.73±1.26 *[a]*
N x T (M+F)	14.30±2.07 [b]	8.35±1.28 [a]	7.31±1.24 [bc]	6.22±2.21 [b]	6.04±1.4 [ab]
T x N (M)		9.01±1.06 *[b]*	8.04±1.37 *[b]*	5.45±1.89	6.70±1.58 *[b]*
T x N (F)		8.30±0.85 *[a]*	6.86±0.67 *[a]*	5.40±0.87	5.01±1.29 *[a]*
T x N (M+F)	13.48±0.97 [b]	8.63±0.99 [a]	7.45±1.21 [c]	5.42±1.43 [ab]	5.83±1.65 [ab]
Sex effect					
Male		8.98±1.41	6.74±1.44 *[B]*	5.05±1.42 *[A]*	6.50±1.50 *[B]*
Female		9.00±1.46	6.52±1.61 *[A]*	5.66±1.85 *[B]*	5.50±1.32 *[A]*

[a, b] Means marked with different letters within each column are significantly different ($P < 0.05$).

[a, b] Significant differences within the breed between males and females ($P < 0.05$).

[A, B] Significant differences within the age between males and females ($P < 0.05$).

SM = age at sexual maturation

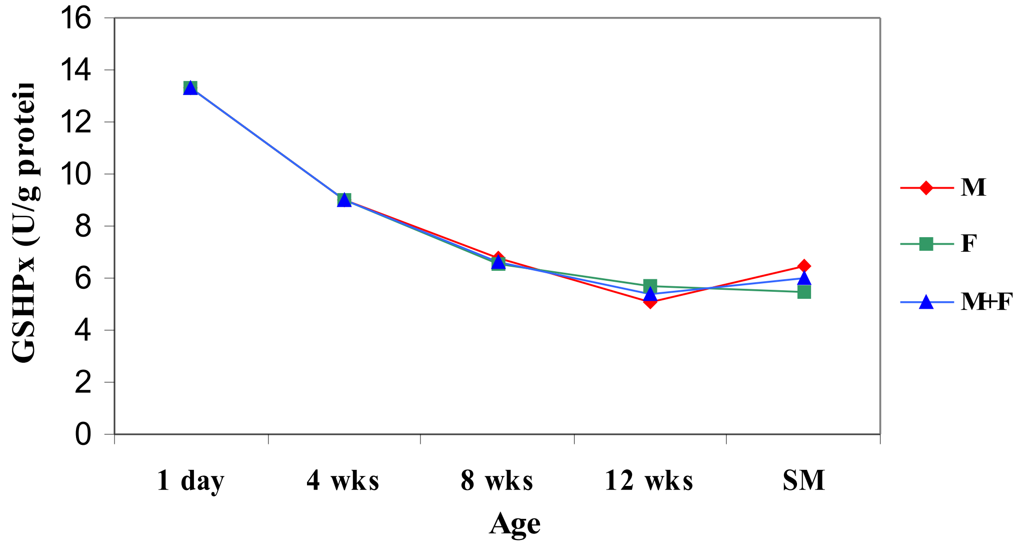


Fig. 15. Blood plasma GSHPx activity of different sex during development
GSHPx (U/g protein
16
14
12
10
8
6
4
2
0
1 day
4 wks
8 wks
12 wks
SM
Age
M
F
M+F

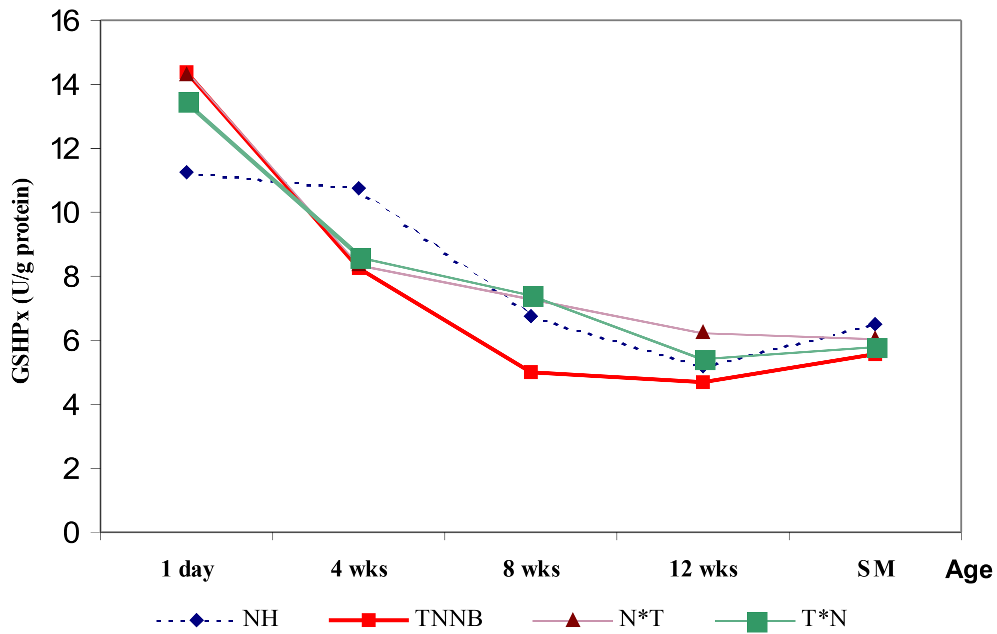


Fig. 16. Blood plasma GSHPx activity of different breeds during development
GSHPx (U/g protein)
16
14
12
10
8
6
4
2
0
1 day
4 wks
8 wks
12 wks
SM
Age
NH
TNNB
N*T
T*N

5. 3. 4. Phenotypic variation of body weight

Means of body weight are given in Table 27. Significant differences in the body weight were found between the two breeds and crosses in all age groups. NH has higher body weight than TNNB in all age groups. The N x T cross had higher body weight than the reciprocal cross in all age groups except at 4 weeks of age. Males had higher body weight than females in all breeds at all age groups except T x N cross at 8 weeks of age (Table 27).

Table 27. Body weight (kg) of different breeds, sex and age groups (mean ± SD)

Breed	4 weeks	8 weeks	12 weeks	SM
NH (M)	0.19±0.04	0.67±0.07 ^{b}	1.30±0.24 ^{b}	2.39±0.24 ^{b}
NH (F)	0.16±0.04	0.48±0.07 ^{a}	1.04±0.18 ^{a}	2.14±0.18 ^{a}
NH (M+F)	0.18±0.04 ^{b}	0.58±0.12 ^{b}	1.16±0.24 ^{b}	2.26±0.24 ^{b}
TNNB (M)	0.12±0.03	0.44±0.12 ^{b}	1.04±0.25 ^{b}	2.14±0.25 ^{b}
TNNB (F)	0.13±0.03	0.33±0.07 ^{a}	0.73±0.16 ^{a}	1.83±0.16 ^{a}
TNNB (M+F)	0.12±0.03 ^{a}	0.39±0.11 ^{a}	0.88±0.26 ^{a}	1.98±0.26 ^{a}
N x T (M)	0.21±0.05	0.62±0.14 ^{b}	1.26±0.19 ^{b}	2.36±0.19 ^{b}
N x T (F)	0.19±0.04	0.54±0.13 ^{a}	1.00±0.22 ^{a}	2.10±0.22 ^{a}
N x T (M+F)	0.20±0.05 ^{b}	0.58±0.14 ^{b}	1.13±0.24 ^{b}	2.23±0.24 b
T x N (M)	0.23±0.05	0.48±0.08 ^{a}	1.33±0.10 ^{b}	2.43±0.10 ^{b}
T x N (F)	0.21±0.02	0.64±0.08 ^{b}	0.91±0.12 ^{a}	2.01±0.12 ^{a}
T x N (M+F)	0.22±0.04 ^{b}	0.56±0.11 ^{b}	1.11±0.24 ^{b}	2.21±0.24 ^{b}
Sex effect				
Male	0.19±0.06	0.55±0.14	1.23±0.23 ^{B}	2.33±0.23 ^{B}
Female	0.17±0.05	0.50±0.14	0.92±0.21 ^{A}	2.02±0.21 ^{A}

$^{a, b}$ Means marked with different letters within each column are significantly different ($P < 0.05$).
^{a,b} Significant differences within the breed between males and females ($P < 0.05$).
$^{A, B}$ Significant differences within the age between males and females ($P < 0.05$).
SM = age at sexual maturation

5. 3. 5. Heterosis and reciprocal effects

Mean heterosis and reciprocal effects as a percentage of the mid-parent values of N x T and T x N crosses for RBC haemolysate, blood plasma and liver GSHPx activity and live body weight are presented in Table 28. Regardless the age effect, the heterosis effect for T x N cross had higher percent than N x T cross for RBC and liver enzyme activity and lower percent for blood plasma. Heterosis as a percentage of the mid-parent values for RBC and blood plasma affected by age as shown in Table 28. Mean heterosis as a percentage of the mid-parent values of N x T and T x N crosses values for live body weight

showed that the heterosis effect was higher for N x T than T x N cross at all ages except at 4 weeks (Table 28). The heterosis effect for live body weight decreased with age for both crosses. Reciprocal differences have high values for all traits as shown in Table 28.

Table 28. Mean heterosis and reciprocal differences as a percentage of the mid-parent values of N x T and T x N crosses of RBC haemolysate, blood plasma (BP) and liver GSHPx activity and live body weight (BW)

Age	Heterosis differences %								Reciprocal differences %			
	N x T				T x N							
	RBC	BP	BW	Liver	RBC	BP	BW	Liver	RBC	BP	BW	Liver
1 day	78	12		-12.3	3	5.5		-27.6	75	6.5		15.4
4 wks	34.5	-12	33.3		-5.5	-9	46.7		40	-3	-13.4	
8 wks	-3	24.5	19.6		8	27	15.5		-11	-2.5	4.1	
12 wks	-19	26.6	10.8		-27	10.4	8.82		8	16	2	
SM	-18	0	5.2		-21	-3.5	4.3		3	3.5	1	
OM	**-3**	**10**			**-11**	**6**			**8**	**4**		

SM = age at sexual maturation
OM = overall mean.

5. 3. 6. Phenotypic correlation between GSHPx activity in RBC haemolysate and blood plasma with body weight

The phenotypic correlations between body weight and RBC haemolysate GSHPx activity at different age of the various breeds had different values (Table 29). Regarding the breed effect, positive correlations were found for NH and TNNB while negative correlations were found for the two crosses. Concerning the age effect, negative correlations were found between BW and RBC GSHPx activity at 4, 8 and 12 weeks of age and positive at sexual maturity. Regardless of breed and age effects, positive correlation was found as shown in Table 29.

The phenotypic correlation between body weight and blood plasma enzyme activity at different age of the various breeds had different values (Table 29). In the case of breed effect, negative correlations were found for all breeds. Concerning the age effect, negative correlations were found between BW and blood plasma GSHPx activity at 8 weeks of age and the correlations were positive at 4, 12 weeks of age and at sexual maturity. The overall of all observation showed that, negative correlation was found between body weight and blood plasma enzyme activity as shown in Table 29.

5. 3. 7. Phenotypic correlation between liver, RBC and blood plasma GSHPx activity

Significant positive correlation was found between blood plasma and RBC GSHPx activity at the age of sexual maturity (0.30; $P \leq 0.01$).

Table 29. Phenotypic correlation between GSHPx activity in RBC haemolysate and blood plasma with body weight of different breeds at different age

	NH	TNNB	N x T	T x N	OM
		RBC *vs.* body weight			
4 weeks	-0.22	0.02	-0.31	-0.49	-0.11
8 weeks	0.27	-0.16	0.07	-0.12	-0.10
12 weeks	-0.14	-0.22	0.04	-0.05	-0.10
SM	0.13	0.15	0.10	0.12	0.12
Overall mean	0.41**	0.10	-0.10	-0.10	0.10
		Blood plasma *vs.* body weight			
4 weeks	-0.12	0.03	-0.35	0.48	0.10
8 weeks	0.29	0.23	-0.28	-0.18	0.33**
12 weeks	-0.05	-0.26	-0.39**	0.05	-0.11
SM	0.08	0.10	0.10	0.39*	0.17
Overall mean	-0.39**	-0.18	-0.42**	-0.43**	-0.31**

*Correlation is significant at 0.05 level.
** Correlation is significant at 0.01 level.
SM = age at sexual maturation
OM = Overall mean

6. DISCUSSION

Several studies pointed out that the expression of antioxidant enzyme 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; Powers et al., 1992; Egaas et al., 1995)*. Especially, genetic background, sex and age differences in antioxidant defense mechanism are of importance from many points of view such as aging, cancer development, toxicology, and environmental health in animals *(Lingaas et al. 1991; Rusting, 1992; Van Lieshout and Peters, 1998; Jang et al., 2001)*.

The objective of this study was to identify sources of variation in GSHPx activity, with potential use as early predictors for indirect selection which may be associated with disease resistance and performance traits. GSHPx activity of different tissues may be candidates for use in indirect selection if they have high variation among breeds, high heritability and are correlated to performance or disease resistance. The existence of genetic variation in the GSHPx activity in blood, liver and RBC of different animals suggests that GSHPx activity is genetically regulated. An attempt was made to assess the relative importance of these factors by comparing GSHPx activity of different chicken breeds and their crosses to obtain some information about the inheritance and collect information also about the possible genetic background of the differences of the GSHPx activity in different tissues. The other purpose was to obtain information about its correlation with some production traits, age and sex at standardised conditions, which has not been reported before in detail in chicken.

6. 1. Genetic differences in GSHPx activity

Environment and diet was the same for all parent genotypes during the whole experiment. Also, eggs were incubated in uniform conditions concerning the Experiment 1. All genotypes were kept at the same environment and given the same diets during the whole period in Experiments 2. and 3. This suggests that the observed differences among breeds likely to be genetic ones. Thus, the results appear to support those of *Langlands et al., (1980), Woolliams et al. (1983) and Fidanci et al (2001), Mizuno (1984), Shen et al. (1992) and Wu and Squires, (1997),* which reported breed differences in the activity of GSHPx in the whole blood of sheep, cattle, goat and chicken, respectively. Also the present results are in agreement with those reported by *Cestnik (1985)* who found significant differences in the GSHPx activity of 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. However, he found that the rank orders of the two breeds were the same during the whole period of investigation, while in our experiment the rank order of the breeds were not consequent. The reason of this latter difference would be the differences in the embryonic development of different genotypes, the differences in growth rate and age at sexual maturity of the different genotypes and he used only two breeds, also our breeds were different.

There is a considerable variation in GSHPx activity of different breeds and this presumably reflects to the differences of the metabolism of a range of different compounds. However, there are few data available on this point. The usefulness of attempting to identify breed variation therefore rests primarily upon whether it provides evidence for genetic involvement. If further studies confirm genetic regulation of enzyme activity, it could be used as selection criteria. The high values of heritability of GSHPx activity provided by *Langlands et al. (1980) and Lingaas et al. (1991)* suggest that such selection would have rapid response.

The poultry industry has a history of using breed crosses and, more recently, strain crosses, mainly to take advantage of heterosis. Two effects, heterosis and reciprocal effects, are important in poultry crosses. Heterosis is the deviation between the cross and midparent means. Reciprocal effects or reciprocal differences are the deviations between the crosses of two parental strains or breeds in which their roles as male or female parents are reversed. Reciprocal effects are the result of sex-linked and maternal effects. Sex–linkage probably account for most important reciprocal effects. Maternal effects are generally considered important for early growth rate and effects of disease mechanisms. Heterosis for some traits is affected by age during early growth and over production cycles. Changes in reciprocal effects over production cycles reflect those of heterosis, but are of less magnitude *(Fairfull, 1990).*

Langlands et al. (1980) reported that heterosis affects partly at the gene expression level of blood GSHPx activity, the heterosis as a percentage of the mid-parent values ranged from -11.3 to 25.5, which is in agreement with our results. The heterosis as a percentage of the mid-parent values ranged from -3 to 78 for red blood cell, from 0 to 27 for blood plasma and from -12.3 to -27.6 for liver glutathione peroxidase activity and from 5.2 to 46.7 for live body weight. The high values of the reciprocal effect on the enzyme activity suggests the possibility of sex-linked and maternal effects on GSHPx activity, but further research is needed to prove this hypothesis.

Our data clearly indicate that, Hungarian breeds (Hungarian White and Hungarian Speckled) have higher enzyme activity in liver and red blood cells than Transylvanian Naked Neck breeds and Plymoth Rock White. Also, New Hampshire breed have the highest liver, red blood cells and blood plasma enzyme activity than all other breeds used in the experiments. The significant differences within breeds also provided evidences of genetic regulation of the enzyme activity, which give an opportunity to make selection within breeds.

These data further suggest that selective breeding of chicken for new strains differing in sensitivity to nutritional selenium deficiency and high tolerance to oxidative stress might be possible. Such strains would be valuable tools in studies of biochemical basis of mode of nutritional action of selenium and in the way metabolism difference of a range of compounds of different breeds.

It is also interesting to note that, given the tissue-specificity and breed-specific developmental profile of chicken glutathione peroxidase expression, it may be argued that genetic regulatory mechanisms may exist that are capable of modulating the expression of enzyme activity. Such regulatory elements are expected to play a vital role in offering protection against oxidative tissue damage.

The present study suggest that it is important to carry out further investigations on the genetic and phenotypic factors influencing other antioxidant enzyme activity as a basis for evaluation of their potential use in the prediction of disease resistance and performance traits. Studies concerning correlation between antioxidant enzyme activity and economically important traits would be important. Further studies are needed to investigate other antioxidant enzymes and inheritance of their expression in chicken during embryonic development.

6. 2. Effect of sex on GSHPx activity

Previous studies have demonstrated sex effect on the GSHPx activity in different tissues of rats, mice and pigs, but there was no available data concerning the sex effect on GSHPx activity in chickens at standard environmental conditions.

Sex differences in liver GSHPx activity during embryonic development were not reported before. Our results indicated that females had higher liver enzyme activity than males during embryonic development and at day-old age in all studied breeds except PRW. Similar results were obtained with GSHPx activity in rat liver, where females had higher activity than males *(Capel and Smallwood 1983; Burk, 1983; Igarashi et al., 1984; Debski et al., 1992; Prohaska and Sunde, 1993; Sachdev and Sunde, 2001).* Also, in mice liver GSHPx activity was higher in females compared to males *(Prohaska and Sunde, 1993).*

Regardless of age and breed effects, red blood cell and blood plasma GSHPx activity was higher in males than in females in Experiments 2 and 3 and females had higher activity than males in Experiment 1 (day-old age). The GSHPx activity in red blood cells was higher in females than in males, but greater in plasma in males of another avian species, Japanase quail *(Godin et al., 1995).*

6. 3. Effect of age on GSHPx activity

In general, studies of variations in glutathione peroxidase activity with increasing age have shown mixed pattern of increases and decreases that are both tissue and species dependent.

Our results showed that, the enzyme activity decreased with age in liver during the embryonic development untill time of hatching, which is in disagreement with some previous findings *(Gaál et al., 1995; Surai, 1999).* Those reported that enzyme activity increased throughout embryonic development, reaching its maximum at time of hatching. This difference may be caused by the different breeds or the differences in selenium content of the maternal diet *(Surai, 2000).*

Our data also indicated that, RBC glutathione peroxidase activity decreased from day-old age untill 12 weeks of age and increased from 12 weeks of age untill the age of sexual maturity and then decreased again till the age of highest egg production. Blood plasma enzyme activity decreased with age from day-old till the age of highest egg production. In agreement with our observation *Godin et al. (1995)* reported that red blood cell and blood plasma GSHPx activity decreased with age in Japanese quail from day-old untill 9 weeks of age. Also, in the case of sheep, erythrocyte GSHPx activity slightly

decreased with age *(Atroshi and Sankari, 1981).* In contrast, blood plasma and red blood cell GSHPx activity increased in porcine blood as effect of ageing *(Jørgensen et al., 1977).*

6. 4. Phenotypic correlation between production traits and GSHPx activity

Few previous work 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 breed, age and tissues. Most of these data are reported for the first time.

Negative correlations were found between GSHPx activity of liver at 14th and 20th but positive correlation at 16th and 18th day of incubation and egg weight 14th, 16th, 18th and 20th day of incubation as well as body weight of day-old chicken. Also negative correlations were found between GSHPx activity of blood plasma while, positive correlations of red blood cell haemolysate and egg weight at 14th, 16th, 18th and 20th day of incubation and body weight of day-old chicken.

Negative correlations were also found between glutathione peroxidase activity of blood plasma, erythrocyte haemolysate and liver and body weight. The same negative correlations were found between GSHPx activity of liver and red blood cells and egg production while, significant positive correlations were between GSHPx activity in blood plasma and egg production. Contrary to our observation, *Squires and Wu (1992)* found that liver GSHPx activity was positively correlated with egg production.

Negative correlations between production traits and GSHPx activity in blood plasma, red blood cell and liver which were obtained in our 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., (1994a)* also *Virág et al (1996)* in rabbit**.**

The significant negative correlation between production traits and GSHPx activity in blood plasma, red blood cell and liver 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.

6. 5. Phenotypic correlations between liver, RBC and blood plasma GSHPx activity

Phenotypic correlation among liver, RBC and blood plasma GSHPx activity are not reported before, our data indicated that, the measure of the enzyme activity in one tissue could be used as a good indicator to the enzyme activity in other tissues based one the high correlation values which obtained in our results but further research need to prove this hypothesis.

6. 6. Suggestions for further research

Concerning the above results several further studies or new research projects can be initiated.

The existence of genetic variation in the GSHPx activities in different chicken breeds suggests that, GSHPx activity is genetically regulated which indicate that GSHPx activity of different tissues may be candidates for use in selection, the correlations between the enzyme activities and production traits which obtained in this study especially the correlations between the enzyme activities at day old with adult body weight and egg production suggest that, this selection could be improve performance and disease resistance.

These data suggest also that selective breeding of chicken for new strains differing in sensitivity to nutritional selenium deficiency and high tolerance to oxidative stress might be possible. Such strains would be valuable tools in studies of biochemical basis of mode of nutritional and physiological action of selenium.

Estimation of heritability of GSHPx activities in different tissues could be valuable tool to study the inheritance of GSHPx activity.

Estimation of genetic correlations between GSHPx activities and production traits also important to know whether this correlations are of phenotypic or genotypic origin.

Artificial challange of these enzyme systems can give further information concerning the real defence against oxidative stress by possibly various level of enzyme induction at different genetic background.

7. SUMMARY

Glutathione peroxidase is a selenium-containing metallo-enzyme, which is part of the biological antioxidant defence mechanism. There are some data about the phenotypic variation of GSHPx enzyme activity in mice, rat, pig, sheep, goat, goose, chicken and rabbit. The present study consists of three experiments conducted to collect information about the possible genetic background of different GSHPx enzyme activities in different breeds and crosses of chickens, and its correlation with some production traits also studying the effect of age and sex on GSHPx activity.

Experiment 1.

Fertilised eggs from eight breeds (Plymouth Rock White, New Hampshire, Hungarian White, Hungarian Speckled, Naked Neck Plymouth, Naked Neck New Hampshire, Transylvanian Naked Neck White, Transylvanian Naked Neck Black) were obtained. Eggs of each breed were weighed and liver samples were taken at 14th, 16th, 18th and 20th days of incubation. GSHPx activity was measured in embryonic liver and in blood plasma, erythrocyte haemolysate and liver of day-old chicken. The sex of embryo and day-old chicken was determined by RAPD-PCR method.

The results showed significant differences in the GSHPx activity of liver among genotypes, but the differences were not consequent during the embryonic development. Age of the embryo had significant effect on liver GSHPx activity, it decreased with age. Females had higher liver, red blood cell and blood plasma activity than males. Negative correlations were found between GSHPx activity of liver at 14th and 20th but positive correlations at 16th and 18th day of incubation and egg weight at 14th, 16th, 18th and 20th day of incubation, as well as body weight of day-old chicken. Also negative correlations were found between GSHPx activity of blood plasma, but it were positive correlations of red blood cell haemolysate and egg weight at 14th, 16th, 18th and 20th days of incubation and body weight of day-old chicken. Significant negative correlations were found between liver enzyme activity at 14th day of incubation with liver enzyme activity (-0.76), with RBC (-0.52) and with body weight (-0.33) of day-old chicken, while significant positive correlation was found for blood plasma (0.76).

Experiment 2.

Blood samples were collected from six genotypes (Plymouth Rock White, Naked Neck Plymouth, Naked Neck New Hampshire, Hungarian Speckled, Transylvanian Naked

Neck White, Hungarian White), aged one-day, 4 weeks, 8 weeks, 12 weeks, at the period of sexual maturity and at the age of the highest egg production. GSHPx activity was measured in blood plasma and erythrocyte haemolysate. The enzyme activity in the liver was also measured at day-old age.

The results showed that there were significant differences in the glutathione peroxidase activity of blood plasma and erythrocyte haemolysate among genotypes in all age groups. The significant differences were not consequent at different age groups. There were also some significant differences in the enzyme activity of liver among different genotypes. Sex also has a significant effect on red blood cell and blood plasma glutathione peroxidase enzyme activity. Negative correlations were found between glutathione peroxidase activity of blood plasma, erythrocyte haemolysate and liver at day-old age and body weight. Negative correlation was found between GSHPx activity of liver and red blood cells and egg production and significant positive correlations were between GSHPx activity in blood plasma and egg production.

Experiment 3.

Glutathione-peroxidase activity was measured in erythrocyte haemolysate and blood plasma at one day, 4, 8 and 12 weeks of age and at the time of sexual maturity and in liver at day-old age of two breeds (New Hampshire and Transylvanian Naked Neck Black) and their crosses (New Hampshire sires crossed Transylvanian Naked Neck Black dames and reciprocal one).

The results showed that there are significant differences in the glutathione enzyme activity of erythrocyte haemolysate, blood plasma and liver among genotypes. The significant differences are not consequent at different age groups in erythrocyte haemolysate and blood plasma. Heterosis plays a role in the expression of enzyme activity. The heterosis as a percentage of the mid-parent values ranged from -3 to 78 for red blood cell, from 0 to 27 for blood plasma and from -12.3 to -27.6 for liver glutathione peroxidase activity and from 5.2 to 46.7 for live body weight. The high values of the reciprocal effect on the enzyme activity suggests the possibility of sex-linked and maternal effects on the enzyme activity. The phenotypic correlation between the enzyme activity and production traits depends on genotype, age and tissue.

8. NEW SCIENTIFIC RESULTS

1. The measure of the phenotypic variation of GSHPx enzyme activity of liver in the embryo's of different chicken genotypes of during embrionic development at standardised conditions.

2. The measure of the phenotypic variation of GSHPx enzyme activity of liver, RBC and blood plasma in different genotypes and their crosses of chickens from day-old age up to the age of the highest egg production at standardised conditions.

3. The measure of the effect of heterosis and reciprocal effect on the GSHPx enzyme activity of liver, RBC and blood plasma and body weight.

4. The measure of the effect of age and sex on GSHPx enzyme activity of liver, RBC and blood plasma during development of chick-embryo and from day-old up to the age of the highest egg production at standardised conditions.

5. The estimation of the correlation among different production traits and GSHPx enzyme activity of liver, RBC and blood plasma.

9. PUBLICATIONS RELATED TO THE SUBJECT OF DISSERTATION

Articles published in scientific periodicals:

1. **Gihan Shaaban,** Mézes, M. and Hidas, A. (2003): Phenotypic variation of glutathione peroxidase activity in different genotypes of different age groups of chicken and its correlation with some production traits. Europ. Poult. Sci. (Arch. Geflügelk). 67 (2), 1-8.

2. **Gihan Shaaban**, Mézes, M. and Hidas, A. (2003): Phenotypic variation in the activities of glutathione peroxidase and their correlation with some production traits in two chicken breeds and their crosses. Europ. Poult. Sci. (Arch. Geflügelk). (Accepted for publication).

3. Mézes M.- Erdélyi M.- **Gihan Shaaban** (2003): Genetics of the glutathione peroxidase enzyme. Acta Biologica Szegediensis. (Accepted for publication).

Papers and abstracts in conference proccedings:

1. **Gihan Shaaban,** Mézes, M. and Hidas, A. (2001): Phenotypic variation of glutathione peroxidase activity in different genotypes of different age groups of chicken. Proc. 2nd Poult. Genetics Symposium, Gödöllõ, pp. 110-111.

2. **Gihan Shaaban,** Mézes, M. and Hidas, A. (2002): Phenotypic variation in the activity of glutathione peroxidase and their correlation with some production traits in two chicken breeds and their crosses. Proc. 11th Europ. Poult. Conference, Bremen, pp. 98. (Abstr.)

3. **Gihan Shaaban,** Mézes, M. and Hidas, A. (2002): Phenotypic variation of glutathione peroxidase activity in different genotypes of chicken and its correlation with production traits. Proc. 53nd Annual Meeting of EAAP, Cairo, Vol. 8. pp. 13. (Abstr.)

4. **Gihan Shaaban,** Mézes, M., Balogh K. and Hidas, A. (2003): Effect of breed and sex on glutathione peroxidase activity in chicken embryo and its correlation with some production traits. V. Magyar Genetikai Kongresszus, Siófok. pp. 116. (Abstr.)

5. **Gihan Shaaban,** Mézes, M. and Hidas, A. (2003): Phenotypic variation in the activity of glutathione peroxidase in blood plasma and red blood cells in four breeds and two crosses of chickens and their correlation with body weight. V. Magyar Genetikai Kongresszus. Siófok. pp. 114. (Abstr.)

Conference presentations:

Mézes M., Erdélyi M. and **Gihan Shaaban** (2002): A glutation peroxidáz genetikája. Szabadgyök Kutatás 2002. konferencia, Szeged.

10. REFERENCES

Aebi, H. and Suter, H. **(1974):** Protective function of reduced glutathione (GSH) against the effect of prooxidative substances and of irradiation in the red cell. In: Flohé, L., Benöhr, H. Ch., Sies H., Waller, H. D. and Wendel, A., eds.: *Glutathione,* pp. 192-201, Georg Thieme, Stuttgart.

Akashi, K.; Miyake, C. and Yokota, A. **(2001):** Citrulline, a novel compatible soute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. *FEBS Lett.* **508,** 438-447.

Allen, R. G. and Venkatraj, V. S. **(1992):** Oxidants and antioxidants in development and differentiation. *J. Nutr.* **122,** 631-635.

Andrewartha, K. A. **(1978):** Victorian Vet. Proc. (Australia) **36,** 42-45.

Arias, I. M.; Fleischner, G.; Kirsch, R.; Mishkin, S. and Gatmaitan, Z. **(1976):** On the structure, regulation, and function of ligandin. In: Arias, I. M. and Jakoby, W. B., eds.: *Glutathione*, pp. 175-188, Raven Press, New York.

Arthur, J. R. **(2000):** The glutathione peroxidases. *Cell. Mol. Life Sci.* **57,** 1825-1835.

Atroshi, F. and Sankari, S. **(1981):** Variation of erythrocyte glutathion peroxidase activity in Finn sheep. *Res. Vet. Sci.* **31,** 267-271.

Aumann, K. D.; Bedorf, N.; Flohe, R.; Schomburg, D. and Flohe, L. **(1997):** Glutathione peroxidase revisited: simulation of the catalytic cycle by computer-assisted molecular modelling. *Biomed. Environm. Sci.* **10,** 136-155.

Avissar, N.; Eisenmann, C.; Breen, J. G.; Horowitz, S.; Miller, R. K. and Cohen, H. J. **(1994a):** Human placenta makes extra-cellular glutathione peroxidase and secretes it into maternal circulation. *Am. J. Phys.* **267,** E68-E76.

Avissar, N.; Ornt, D. B.; Yagil, Y.; Horowitz, S.; Watkins, R. H. and Kerl, E. A. **(1994b):** Human kidney proximal tubules re the main source of plasma glutathione peroxidase. *Am. J. Phys.* **266,** C367-C375.

Bakoss, L. **(1931):** Farm poultry breeding. 2nd ed. Csáthy Ferenc Egyetemi Kõnyv-kereskedés és Irodalmi Vállalat Rt., Budapest-Debrecen. (in Hungarian).

Barp, J.; Araujo, A. S.; Fernandes, T. R.; Rigatto, K. V.; Liesuy, S.; Bello-Klein A. and Singal, P. **(2002):** Myocardial antioxidant and oxidative stress changes due to sex hormones. *Braz. J. Med. Biol. Res.* **35,** 1075-1081.

Behne, D.; Hofer, T.; Berrswordt, W. R. and Elger, W. **(1982):** *J. Nutr.* **112,** 1682-1687.

Behne, D.; Duk, M. and Elger, W. **(1986):** Se content and glutathione peroxidase activity in the testis of the maturing rat. *J. Nutr.* **116,** 1442-1447.
Bell, R. and Draper, H. **(1976):** GSHPx activity and glutathione concentration in genetical dystrophic mice. *Proc. Soc. Exp. Biol. Med.* **152,** 520-523.

Bermano, G.; Nicol, F. J.; Dyer, A.; Sunde, R. A.; Beckett, G. J.; Arthur, J. R. and Hesketh, J. E. **(1995):** Tissue-specific regulation of selenoenzyme gene expression during selenium deficiency in rats. *Biochem. J.* **311,** 425-430.

Binda, D.; Nicod, L.; Viollon-Abadie, C.; Rodriguez, S.; Berthelot, A.; Coassolo, P. and Richert, L. **(2001):** Strain difference (WKY, SPRD) in the hepatic antioxidant status in rat and effect of hypertension (SHR, DOCA). *Ex vivo and in vitro* data. *Mol. Cell. Biochem.* **218,** 139-146.

Borras, C.; Sastre, J.; Garcia-Sala, D.; Lloret, A.; Pallardo, F. V. and Vina, J. **(2003):** Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Rad. Biol. Med.* **34,** 1-7.

Bottje, W. G. and Wideman, R. F. **(1995):** Potential role of free radicals in the pathogenesis of pulmonary hypertension syndrome. *Poult. Avian Bio. Rev.* **6,** 211-231.

Brannan, T. S.; Maker, H. S. and Weiss, C. **(1981):** Developmental study of rat brain glutathione peroxidase and glutathione reductase. *Neurochem. Res.* **61,** 41-45.

Bui, L. M.; Keen, C. L. and Dubick, M. A. **(1995):** Comparative effects of a 6-week nicotine treatment on blood pressure and components of the antioxidant system in male spontaneously hypertensive (SHR) and normotensive Wistar Kyoto (WKY) rats. *Toxicology.* **98,** 57-65.

Bunk, M. J.and Combs, G. F. **(1981):** Relationship of selenium-dependent glutathione peroxidase activity and nutritional pancreatic atrophy in selenium-deficient chicks. *J. Nutr.* **111,** 1611-1620.

Burk, R. F. **(1983):** Glutathione peroxidase. *Ann. Rev. Nutr.* **3,** 53-70.

Burk, R. F.; Nishiki, K.; Lawrence, R. A. and Chance, B. **(1978):** Peroxide removal by Selenium -dependent and selenium-indepentent glutathione peroxidases in hemo-globin-free perfused rat liver. *J. Biol. Chem.* **253,** 43-46.

Capel, I. D. and Smallwood, A. E. **(1983):** Sex differences in the glutathione peroxidase activity of various tissues of the rat. *Res. Commun Chem. Pathol Pharmacol.* **40,** 367-378.

Cestnik, V. **(1985):** Glutathione peroxidase activity in embryonal development of chicken. *Veterinarstvo.* **22,** 187-193.

Chance, B.; Greenstein, D. S. and Roughton, F. J. W. **(1952):** The mechanism of catalase action. I. Steady-state analysis. *Arch. Biochem. Biophys.* **37,** 301-321.

Cheng, W. H.; Valentine, B. A. and Lei, X. G. **(1999):** High levels of dietary vitamin E do not replace cellular glutathione peroxidase in protecing mice from acute oxidative stress. *J. Nutr.* **129,** 1951-1957.

Chester, J. K. and Arthur, J. R. **(1988):** Early biochemical defects caused by dietry trace elements deficiencies. *Nutr. Res. Rev.* **1,** 39-56.

Chiaradia, E.; Gaiti, A.; Scaringi, L.; Cornacchione, P.; Marconi, P. and Avellini, L. **(2002):** Antioxidant systems and lymphocyte proliferation in the horse, sheep and dog. *Vet. Res.* **33,** 661-668.

Chiu, D.; Fletcher, B.; Stults, F.; Zakowski, J. and Tappel, A. L. **(1975):** Properties of selenium-glutathione peroxidase. *Fed. Proc.* **34,** 925 (Abstr. 3996).

Chow, C. K. and Tappel, A. L. **(1972):** An enzymatic protective mechanism aginst lipid peroxidation damge to lungs of ozone-exposed rats. *Lipids*. **7,** 518-524.

Christ-Hazelhof, E.; Nugteren, D. H. and Van Dorp, D. A. **(1976):** Conversions of prostaglandin endoperoxides by glutathione S-transferases and serum albumins. *Bio-chim. Biophys. Acta.* **450,** 450-461.

Chu, F. F.; Doroshow, J. H. and Esworthy, R. S. **(1993):** Expression, characterisation and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J. Biol. Chem.* **268,** 2571-2576.

Condell, R. A. and Tappel, A. L. **(1983):** Evidence for suitability of GSHPx as a protective enzyme: Studies of oxidative damage, denaturation, and proteolysis. *Arch. Biochem. Biophys*. **223,** 407-416.

Corbera, J. A.; Gutierrez, C.; Morales, M.; Montel, A. and Montoya, J. A. **(2001):** Assessment of blood glutathione peroxidase activity in the dromedary camael. *Vet. Res.* **32,** 185-191.

Cunningham, D. L.; Combs, G. F.; Saroka, J. A. and LaVorgna, M. W. **(1987):** Response to divergent selection for early growth of chickens fed a diet deficient in selenium. *Poult. Sci.* **66,** 209-214.

Dahle, L. K.; Hill, E. G. and Hollmann, R. T. **(1962):** The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methyl esters. *Arch. Biochim. Biophys*. **98**, 253-261.

Dear, T. N.; Campbell, K. and Rabbits, T. H. **(1991):** Molecular cloning of putative odorant-binding and odorant-metabolizing proteins. *Biochem. J.* **285,** 863-870.

Debski, B.; Zarski, T. and Milner, J. **(1992):** The influence of age and sex on selenium distribution and glutathione peroxidase activity in plasma and erythrocytes of selenium adequate and supplemented rats. *J. Physol. Pharmacol.* **3,** 299-306.

Duncan, D. B. **(1955):** The Multiple Range and Multiple F Test. *Biometrics.* **11,** 1-42.

Edfors-Lilja, L.; Petersson, H. and Gahne, B. **(1986):** Performance of pigs with or without the intestinal receptor for *Escherichia coli* K88. *Anim. Prod.* **42,** 381-387.

Egaas, E.; Falls, F. G. and Dauterman, W. C. **(1995):** A study of gender, strain and age differences in mouse liver glutathione-S-transferase. *Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol.* **110,** 35-40

Egglestone, L. V. and Krebs, H. A. **(1974):** Regulation of the pentose phosphate cycle. *Biochem. J.* **183,** 425-435.

El Mouatassim, S.; Guerin, P. and Menezo, Y. **(2000):** Mammalian oviduct and protection against free oxygen radicals: expression of genes encoding antioxidant enzymes in human and mouse. European J. *Obs. Gyneco . Reprod. Biol.* **89,** 1-6.

Epp, O.; Ladenstein, R. and Wendel, A. **(1983):** The refined structure of the selenoenzyme glutathione peroxidase at 0.2 nm resolution. *Eur. J. Bioch.* **133,** 51-69.

Evans, P. and Halliwll, B. **(2001):** Micronutrients: oxidant/antioxidant status. *Br. J. Nutr.* **85** (suppl 2): S67.

Fairfull, R. W. **(1990):** Heterosis. In: Crawford, R.D. ed.: *Poultry Breeding and Genetics*, pp. 913-933, Elsevier Amsterdam.

Fang, Y. Z. **(1991):** Effect of ionizing radiation on superoxide dismutase in vitro and *in vivo.* In: Fang, Y. Z. ed: *Advances in Free Radical Biology and Medicine.* Atomic Energy Press, Beijing. Vol **1,** 1-18.

Fang, Y. Z. **(2002):** Free radicals and nutrition. In: Fang Y. Z., Zheng, R. L., eds: *Theory and application of free radical biology*. pp.647 Beijing. Scientific Press.

Fantel, A. G. **(1996):** Reactive oxygen species in developmental toxicity: Review and hypothesis. *Teratology.* **53,** 196-217.

Fidanci, U. R.; Turgay, F.; Zengin, S.; Kargin, F.; Celik, S. and Tasdemir, U. **(2001):** The effect of genotype on the antioxidative metabolism in Angora goats. *Turkish J. Vet. Anim. Sci.* **25,** 975-981.

Finley, J. W. and Kincaid, R. L**. (1991):** Effect of sex and time of sampling on selenium and glutathione peroxidase activity in tissues of mature rats. *Biol. Trace Elem. Res.* **29,** 181-191.

Flohé, L. **(1976):** Role of selenium in hydroperoxide metabolism. *Proc. Symp. Selenium-tellurium in the environment.* pp. 138-157, Industrial Health Foundation, Inc., Pittsburgh.

Flohé, L. **(1978):** Ciba Foundation Symposium, No. 65. pp. 95-122.

Flohé, R. **(1999):** Tissue-secific functions of individual glutathione peroxidases. *Free Rad. Biol. Med.* **27,** 951-965.

Flohé, L. and Günzler, W. A. **(1974):** Glutathione peroxidase. In: Flohé, L.; Benöhr, H. Ch.; Sies H.; Waller, H. D. and Wendel, A. eds.: *Glutathione*, pp. 132-145, Georg Thieme, Stuttgart.

Flohé, L. and Menzel, H. **(1971):** The influence of glutathione upon light-induced high-amplitude swelling and lipid peroxide formation of spinach chloroplasts. *Plant Cell Physiol.* **12,** 325-333.

Flohé, L. and Schlegel, W. **(1971):** Glutathione peroxidase, IV. Intrazellulare Verteilung des Glutathione peroxidase- Systems in der Rattenleber. *Hoppe Seyler's Z. Physiol. Chem.* **352,** 1401-1410.

Flohé, L. and Zimmermann, R. **(1970):** The role of GSHPx in protecting the membrane of rat liver mitochondria. *Biochim. Biophys. Acta* **223,** 210-213.

Flohé, L. and Zimmermann, R. **(1974):** GSH-induced high-amplitude swelling of mitochondria. In: Flohé, L.; Benöhr, H. Ch.; Sies H.; Waller, H. D. and Wendel, A., eds.: Glutathione, pp. 245-260, Georg Thieme, Stuttgart.

Flohé, L.; Eisele, B. and Wendel, A. **(1971a):** Glutathione-peroxidase, I. Reindarstellung and Molekular gewichtsb estimmungen. *Hoppe Seyler's Z. Physiol. Chem.* **352,** 151-158.

Flohé, L.; Günzler, W. A, and Ladenstein, R. **(1976):** Glutathione peroxidase. In: Arias, I. M. and Jakoby, W. B., eds.: Glutathione, pp. 115-138, Raven Press, New York.

Flohé, L.; Günzler, W. A. and Schaich, E. **(1972):** Glutathione peroxidase, V. The kinetic mechanism. *Hoppe Seyler's Z. Physiol. Chem.* **353,** 987-999.

Flohé, L.; Günzler, W.; Jung, G.; Schaich, E. and Schneider, F. **(1971b):** Glutathione peroxidase, II. Substratspezifität und Hemmbarkeit durch Substratanaloge. *Hoppe Seyler's Z. Physiol. Chem.* **352,** 159-169.

Flohé, L.; Schaich, E.; Voelter, W. and Wendel, A. **(1971c):** Glutathione peroxidase, III. Spektrate Charakteristika und Versuche zum Reaktions mechanismus. *Hoppe Seyler's Z. Physiol. Chem.* **352,** 170-180.

Forstrom, J. W.; Zakowski, J. J. and Tappel, A. L. **(1978):** Identification of the catalytic site of rat liver GSHPx as selenocysteine. *Biochemistry.* **17,** 2639-2644.

Freeman, B. M. and Vince, M. A. **(1974):** *Development of the avian embryo*. pp. 249-260. Chapman and Hall, London.

Fridovich, I. **(1999):** Fundamental aspects of reactive oxygen species, or what is the matter with oxygen?. *Ann. N. Y. Acad. Sci.* **893,** 13-18.
Günzler, W. A. **(1974):** Glutathione peroxidase, Kristallisation, Selengehalt, Aminosaure-zusammensetzung und Modellvorstellungen zum Reaktionsmechnismus, Dissertation, Tübingen.

Günzler, W. A.; Vergin, H.; Müller, I. and Flohé, L. **(1972):** Glutathione peroxidase, VI. Die reaktion der GSHPx mit verschiedenen Hydroperoxiden. *Hoppe Seyler's Z. Physiol. Chem.* **353,** 1001-1004.

Gaál, T.; Mézes, M.; Noble, R.; Dixon, C. J. and Speake, B. K. **(1995):** Development of antioxidant capacity in tissues of the chick embryo. *Comp. Biochem. Physiol.* **112B,** 711-716.

Ganther, H. E.; Hafeman, D. G.; Lawrence, R. A.; Serfass, R. E. and Hoekstra, W. G. **(1976):** Selenium and GSHPx in health and disease: A review. In: Prasad, A. ed.: *Trace Elements in Human Health and Disease*, Vol. II., Academic Press, New York.

Ghyselinck, N. B. and Dufaure, J. P. **(1990):** A mouse cDNA sequence for epididymal androgen-regulated protein related to glutathione peroxidase. *Nucl .Acids Res.* **18,** 7144-7149.

Ghyselinck, N. B.; Jimenez, C. and Dufaure, J. P. **(1991):** Sequence homology of androgen-regulated epididymal proteins with glutathione peroxidase in mice. *J. Reprod. Fertil.* **93,** 461-466.

Gilbert, D. L. **(2000):** Fifty years of radical ideas. *Ann. N. Y. Acad. Sci.* **899,** 1-14.

Gille, G. and Sigler, K. **(1995):** Oxidative stress and living cells. *Folia Microbiol.* **40,** 131-152.

Glass, G. A. and Gershon, D. **(1984):** Decreased enzymic protection and increased sensitivity to oxidative damage in erythrocytes as a function of cell and donor aging. *Biochem. J.* **218,** 531-537.

Godin, D.; Garnett, M.; Cheng, K. and Nichols, C. **(1995):** Sex-related alterations in antioxidant status and susceptibility to atherosclerosis in Japanease quail. *Can. J. Cardiol.* **10,** 945-951.

Gonzalez, M. M; Nadrid, R. and Arahuetes, R. M. **(1995):** Physiological changes in antioxidant defences in fetal and neonatal rat liver. *Reprod. Fertil. Dev.* **7,** 1375-1380.

Grossmann, A. and Wendel, A. **(1983):** Non-reactivity of the selenoenzyme glotathione peroxidase with enzymically hydroperoxidised phospholipids. *Eur. J. Bioch.* **135,** 549-552.

Gumuslu, S.; Bilmen, S.; Korgun, D. K.; Yargicoglu, P. and Agar, A. **(2001):** Age-related changes in antioxidant enzyme activity and lipid peroxidation in lungs of control and sulfur dioxide exposed rats. *Free Radic. Res.* **34,** 621-627.

Gunther, T.; Hollrieglm, V. and Vormann, J. **(1993):** Perinatal development on iron and antioxidant defence system. *J. Trace Elem. Electrol. Health and Dis.* **7,** 47-52.

Gutteridge, J. M. C. and Halliwell, B. **(1990):** The measurement and metabolism of lipid peroxidation in biological systems. *Trends Biochem. Sci.* **15,** 129-135.

Haber, F. and Weiss, J. **(1934):** The catalytic decomposition of hydrogen peroxide by iron salts. *Proc. R. Soc. Lond.* **147,** 332-351.

Hafeman, D. G. and Hoekstra, W. G. **(1977):** Lipid peroxidation *in vivo* during vitamin E and selenium deficiency in the rat as monitored by ethane evolution. *J. Nutr.* **107,** 666-672.

Hall, L.; Williams, K.; Perry, A. C.; Frayne, J. and Jury, J. A. **(1998):** The majority of human glutathione peroxidase type 5 (GPX-5) transcripts are incorrectly spliced: implications for the role of GPX-5 in the male reproductive tract. *Biochem. J.* **333,** 5-9.

Halliwell, B. **(1994a):** Free radicals, antioxidants, and human disease: curiosity, cause, or consequence ?. *Lancet.* **344,** 721-724

Halliwell, B. **(1994b):** Free radicals and antioxidants: A personal view. *Nutr. Rev.* **52,** 253-265.

Halliwell, B., and Gutteridge, J. M. C. **(1984):** Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **219,** 1-14.

Halliwell, B., and Gutteridge, J. M. C. **(1999):** Free radical in biology and medicine. 3rd. ed., Oxford University Press. Oxford.

Halpin, K. M. and Baker, D. H. **(1984):** Selenium deficiency and transsulfation in the chick. *J. Nutr.* **114,** 606-612.

Harman, D. **(1956):** Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11,** 298-300.

Harman, D. **(1988):** Free radicals in aging. *Mol. Cell Biochem.* **84,** 155-161.

Harris, E. D. **(1992):** Regulation of antioxidant enzymes. *J. Nutr.* **122,** 627-630.

Hayes, J. D. and Mclellan, L. I. **(1999):** Glutathione and glutathione-dependent enzymes represent a coordinately regulated defence against oxidative stress. *Free Radic. Res.* **31,** 273-300.

Hayes, J. D. and Strange, R. C. **(1995):** Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radic. Res.* **22,** 193-207.

Hidas, A. and Edvi, M. E. **(2001):** Sex determination with RAPD markers. *2nd Poultry Genetics Symposium, Gödöllő, Hungary.* pp. 89-90.

Hoekstra, W. G. **(1976):** Selenium. In: W. G. Hoekstra, J. W. Suttie, H. E. Ganther, W. M. Mertz, eds.: *Trace Element Metabolism in Animals*, Vol. 2., pp. 61-77. University. Park Press, Baltimore.

Hogg, N. **(1998):** Free radical in disease. *Semin. Reprod. Endocrinol.* **16,** 241-248.

Hong, H. and Johnson, P. **(1995):** Antioxidant enzyme activity and lipid peroxidation levels in exercised and hypertensive rat tissues. *Int. J. Biochem. Cell. Biol.* **27,** 923-931.

Howie, A. F.; Walker, S. W.; Akesson, B.; Arthur, J. R. and Beckett, G. J. **(1995):** Thyroidal extracellular glutathione peroxidase: a potential regulator of thyroid-hormone synthesis. *Biochem. J.* **308,** 713-717.

Hull, S. J. and Scott, M. L. **(1976):** Studies on the changes in reduced glutathione of chick tissues during onset and regression of nutritional muscular dystrophy. *J. Nutr.* **106,** 181-191.

Hurley, W. L. and Doane, R. M. **(1989):** Recent developments in the roles of vitamins and minerals in reproduction. *J. Dairy Sci*. **72,** 784-804.

Ichinose, T.; Arakawa, K.; Shimojo, N. and Sagai, M. **(1988):** Biochemical effects of combined gases of nitrogen dioxide and ozone. II. Species differences in lipid peroxides and antioxidative protective enzymes in the lungs. *Toxicol. Lett.* **42,** 167-176.

Ichinose, T.; Suzuki, A. K.; Tsubone, H. and Sagai, M. **(1982):** Biochemical studies on strain differences on mice in the susceptibility to nitrogen dioxide. *Life Sci.* **18,** 1963-1972.

Igarashi, T.; Satoh,T.; Ono, S.; Iwashita, K.; Hosokawa, M.; Ueno K. and Kitagawa, H. **(1984):** Effect of steroidal sex hormones on the sex-related differences in the hepatic activity of gamma-glutamyl transpeptidase, glutathione S-transferase and glutathione peroxidase in rats. *Res. Commun Chem. Pathol Pharmacol.* **45,** 225-232.

Ignarro, L. J.; Cirino, G.; Casini, A. and Napoli, C. **(1999):** Nitric oxide as a signaling molecule in the vascular system: An overview. *J. Cardiovasc. Pharmacol.* **34,** 879-885.

Ip, C. **(1981):** Prophylaxis of mammary neoplasia by Se supplementation in the initiation and promotion phases of chemical carcinogenesis. *Cancer Res.* **41,** 4386-4390.

Iqbal, M.; Cawthon, D.; Beers, K.; Wideman, R. F. and Bottje, W. G. **(2002):** Antioxidant enzyme activity and Mitochondrial fatty acids in Pulmonary Hypertension Syndrome (PHS) in broilers. *Poult. Sci.* **81,** 252-260.

Iqbal, M.; Cawthon, D.; Wideman,J. R. and Bottje, W. G. **(2001a):** Lung mitochondrial dysfunction in pulmonary hypertension syndrome I. Site specific defects in electron transport chain. *Poult. Sci.* **80,** 485-495.

Iqbal, M.; Cawthon, D.; Wideman, J. R. and Bottje, W. G. **(2001b):** Lung mitochondrial dysfunction in pulmonary hypertension syndrome II. Inability to improve function with repeated addition of ADP. *Poult. Sci.* **80,** 656-665.

Jørgensen, F. P.; Hyldaard, J. and Moustgaard, J. **(1977):** Glutathione peroxidase activity in porcine blood. *Acta Vet. Scand.* **18,** 323-334.

Jackson, M. J. **(1999):** An overview of methods for assessment of free radical activity in biology. *Proc. Nutr. Soc.* **58,** 1001-1010.

Jang, I. E.; Kabryong, C. and Jungsik, C. **(2001):** Effect of age and strain on small intestinal and hepatic antioxidant defense enzymes in Wistar and Fisher 344 rats. *Mech. Ageing Develop.* **122,** 561-570.

Jang, I.; Jung, K. and Cho, J. **(1998):** Age-related changes in antioxidant enzyme activity in the small intestine and liver from Wistar rats. *Exp. Anim.* **4,** 247-252.

Kalytka, V. V. and Donchenko, H. V. **(1995):** The antioxidant system and lipid peroxidation in chickens during postnatal ontogenesis. *Ukrainian Bioch. J.* **67**, 80-85.

Kelso, K. A.; Cerolini, S.; Noble, R. C.; Sparks, N. H. and Speake, B. K. **(1996):** Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. *J. Reprod. Fertil.* **106,** 201-206.

Khan, M. Z.; Szarek, J.; Marchaluk, E.; Macig, A. and Bartlewski, P. M. **(1995):** Effect of concurrent administration of monensin and Se on RBC GSHPx activity and liver Se concentration in broiler chickens. *Biol. Trace Elem. Res.* **49,** 129-138.

Knapen, M. F.; Zusterzeel, P. L.; Peters, W. H. and Steegers, E. A. **(1999):** Glutathione and glutathione-related enzymes in reproduction. A review. *Eur. J. Obst. Gynecol. Reprod .Biol.* **82,** 171-184.

Knight, J. A. **(1998):** Free radicals: Their history and current status in aging and disease. *Ann. Clin. Lab. Sci*. **28**, 331-345.

Kosower, E. M. and Kosower, N. S. **(1974):** Manifestation of changes in the GSH-GSSG status of biological systems. In: Flohé, L., Benöhr, H. Ch., Sies H., Waller, H. D. and Wendel, A., eds.: Glutathione, pp. 276-287, Georg Thieme, Stuttgart.

Kosower, N. S. and Kosower, E. M. **(1976):** Functional aspects of glutathione disulfide and hidden forms of glutathione. In: Arias, I. M. and Jakoby, W. B., eds.: Glutathione, pp. 159-172, Raven Press, New York.

Lander, H. M. **(1997):** An essential role for free radicals and derived species in signal transduction. *FASEB J.* **11,** 118-123.

Langlands, J. P.; Bowles, J. E.; Donald, G. E.; Ch'ang, T. S.; Evans, R.; Hearnshaw, H. and Post, T. B. **(1980):** Genotype as a source of variation in selenium concentration and glutathione peroxidase activity of whole blood from grazing sheep and cattle. Australian *J. Agric. Res.* **31,** 839-848.

Lass, A.; Suessenbacher, A.; Wolkart, G.; Mayer, B. and Brunner, F. **(2002):** Functional and analytical evidence for scavenging of oxygen radicals by L- arginine. *Mol Pharmacol.* **61,** 1081-1088.

LaVronga, M. W. and Combs, G. F. **(1982):** Evidence of a hereditary factor affecting the chick's response to uncomplicated selenium deficiency. *Poult. Sci.* **62,** 164-168.

Lawler, J. M.; Barnes, W. S.; Wu, G.; Song, W. and Demaree, S. **(2002):** Direct antioxidant properties of creatine. *Biochem Biophys Res. Commun.* **290,** 47-52.

Lawrence, R. A. and Burk, R. F. **(1976):** Glutathione peroxidase activity in selenium deficient rat liver. *Biochem. Biophys. Res. Commun.* **71,** 952-956.

Lingaas, F.; Brun, E. and Froslie, A. **(1991):** Estimates of heritability for selenium and glutathione peroxidase levels in pigs. *J. Anim. Breed. Genet.* **108,** 48-53.

Lingaas, F.; Brun, E.; Havre, G.; Froslie, A.; Aarskaug, T. and Vangen, O. **(1992):** Biochemical blood parameters in pigs. 1. Repeatability and effects of breed, litter number and sampling time. *J. Anim. Breed. Genet.* **109,** 221-230.

Little, C. and O'Brien, P. J. **(1968):** An intracellular GSH-peroxidase with a lipid peroxide substrate. *Biochem. Biophys. Res. Commun.* **31,** 145-150.

Lopez-Torres, M.; Perez-Campo, R. and Barja de Quiroga, G. **(1991):** Aging in brown fat: Antioxidant defenses and oxidative stress. *Mech. Aging Dev.* **59,** 129-137.

Lowry, D. H.; Rosenbrough, N. J.; Farr, A. L. and Randall, A. J. **(1951):** Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265-272.

Machilin, L. J. and Bandito, A. **(1987):** Free radical tissue damage: protective role of anti-oxidant nutrients. *FASEB J.* **1,** 441-445.

Mahan, D. **(1999):** Organic selenium: using nature's model to redefine selenium supplementation for animals. In: Lyons, T. P., Jaques, K. A. eds.: *Biotechnology in the feed industry.* Proc. Alltech 15th Annual Symp., pp. 523-535, Nottingham University Press, Nottingham.

Mariorino, M.; Aumann, K. D.; Flohe, R.; Doria, D.; Vandenheuval, J. and McCarthy, J. **(1995):** Probing the presumed catalytic triad of selenium-containing peroxidases by mutational analysis of phospholipid hydroperoxide glutathione peroxidase (PHGPx). *Biol. Chem. Hoppe Seyler.* **376,** 651-660.

Mairorino, M.; Aumann, K. D.; Flohe, R.; Doria, D.; Vandenheuvel, J. and McCarthy, J. **(1998):** Probing the presumed catalytic triad of a selenium-containing peroxidase by mutational analysis. *Z. Ernahrungswiss.* **37,** 118-121.

Maiorino, M.; Thomas, J. P.; Girotti, A. W. and Ursini, F. **(1991):** Reactivity of phospholipid hydroperoxide glutathione peroxidase with membrane and lipoprotein lipid hydroperoxides. *Free. Rad. Res. Comm.* **12,** 131-135.

Maser, R. L.; Magenheimer, B. S. and Calvet, J. P. **(1994):** Mouse plasma glutathione peroxidase: cDNA sequence analysis and renal proximal tubular expression and secretion. *J. Biol. Chem.* **269,** 27066-27073.

Matkovics, B.; Szabó, L. and Varga, Sz. I. **(1988):** Determination of enzyme activity in lipid peroxidation and glutathione pathways (In Hungarian). *Laboratóriumi Diagnosztika.* **15,** 248-250.

McCay, P. B.; Gibson, D. D.; Fong, K. L. and Hornbrook, K. R. **(1976):** Effect of GSHPx activity on lipid peroxidation in biological membrances. *Biochim. Biophys. Acta.* **431,** 459-468.

McCord, J. M. **(2000):** The evolution of free radicals and oxidative stress. *Am. J. Med.* **108,** 652-659.

McDowell, L. R. **(2000):** Reevaluation of the metabolic essentiality of the vitamins. *Asian-Australian J. Anim. Sci.* **13,** 115-125.

Mézes, M. and Barta, M. **(1995):** Genetic differences of liver GSHPx activity in goose. Proc. SFRR Summer Meeting, Budapest (Abstr. 27).

Mézes, M.; Eiben, Cs. and Virág, Gy. **(1994a):** Determination of glutathione peroxidase enzyme activity in blood plasma, red blood cells and liver of rabbits. Correlation between the enzyme activity and some production traits (In Hungarian). Proc. 6th Rabbit Breeding Day, PATE, Kaposvár, pp. 121-126.

Mézes M.; Matkovics, B. Sz.; Varga, I.; DoQuai Hai, K.; Barta, M.; Vinczer, P. and Béres, Jr. **(1994b):** Effect of mineral composition (Béres drops plus) on lipid peroxide and antioxidant status of healthy and lipid peroxide loaded rabbits. Proc. 6th Int. Trace Element Symp., Budapest , pp. 301-316.

Mézes M.; Papp, Z. and Kustos, K. **(1993):** Changes of lipid peroxide and antioxidant status of blood in rabbit does kept at different environmental temperature (In Hungarian) Proc. 5th Rabbit Breeding Day, PATE, Kaposvár, pp. 53-59.

Mézes M.; Pusztai, A. and Virág, Gy. **(1986):** Effect of postnatal development and chronic enteritis on lipid peroxidation and vitamin E content of blood of rabbits. Proc. 3rd. Int. Coll. The Rabbit as a Model Animal and Breeding Object. Section II., Wilhelm-Pieck Universitat, Rostock pp. 162-166.

Mézes, M. and Sályi, G. **(1994):** Effect of acute selenium toxicosis on the lipid peroxide status and the glutathione system of broiler chickens. *Acta Vet. Hung.* **42,** 459-463.

Mézes, M.; Surai, P.; Sályi, G.; Speake, B. K.; Gaál, T. and Maldjian, A. **(1997):** Nutritional metabolic diseases of poultry and disorders of the biological antioxidant defence system. *Acta Vet. Hung.* **45,** 349-360.

Mézes, M.; Szalay, I. and Vas, E. **(1989):** Glutathione metabolism enzymes as possible markers for goose liver selection. Proc. Int. Symp. Current Problems of Avian Genetics. Smolenice pp.114-118.

Mézes M.; Virág, Gy.; Barta, M.; Bersényi, A. and Nofal, R. **(1996):** Peroxide intake affects the relationship between glutathione peroxidase activity and some production parameters in rabbit. Proc. 6th World Rabbit Congr., Toulouse, Vol. 2., pp. 203-208.

Michiels, C.; Raes, M.; Toussaaint, O. and Remacle, J. **(1994):** Importance of selenium glutathione peroxidase, catalase and Cu/Zn-SOD. for cell survival against oxidative stress. *Free Radical Bio. Med.* **17,** 235-248.

Mills, G. C. **(1957):** Hemoglobin catablism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J. Biol. Chem.* **229,** 189-197.

Mizuno, Y. **(1984):** Changes in superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity and thiobarbituric acid-reactive products levels in early stages of development in dystrophic chickens. *Exp. Neurol.* **84,** 58-73.

Montellano, P. **(1986):** Cytochrome P-450. In: Ortiz de Montellano, P. ed.: Structure, Mechanism and Biochemistry. pp. 217-273. Plenum, New. York.

Morrissey, P. A. and O' Brien, N. M. **(1998):** Dietary antioxidant in human health and disease. *Intern. Dairy J.* **8,** 463-472.

Mote, P. L.; Grizzle, J. M.; Walford, R. L. and Spindler, S. R. **(1991):** Influence of age and caloric restriction on expression of hepatic genes for xenobiotics and oxygen metabolising enzymes in the mouse. *J. Geronotol.* **3,** 95-100.

Muradian, K. K.; Utko, N. A.; Fraifeld, V.; Mozzhukhina, T. G.; Pishel, I. N. and Litoshenko, A. Y. **(2002):** Superoxide dismutase, catalase and glutathione peroxidase activity in the liver of young and old mice: linear regression and correlation. *Arch. Gerontol. Geriatrics.* **35,** 205-214.

Nagababu, E.; Chrest, F. J. and Rifkind, J. M. **(2003):** Hydrogen- peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. *Biochim. Biophys. Acta.* **25499,** 1-7.

Necheles, T. F. **(1974):** The clinical spectrum of glutathione-peroxidase deficiency. In: Flohé, L., Benöhr, H. Ch., Sies H., Waller, H. D. and Wendel, A., eds.: Glutathione, pp. 173-180, Georg Thieme, Stuttgart.

Nijhoff, W. A. and Peters, W. H. M. **(1992):** Induction of rats hepatic and intestinal glutathione-S-transferases by dietary butyrated hydroxyanisole. *Bioch. Pharmacol.* **3,** 596-600.

Noble, R. C. and Cocchi, M. **(1990):** Lipid metabolism in the neonatal chicken. *Progr. Lipid Res.* **29,** 107-140.

Ohhira, M.; Ono, M.; Ohhira, M.; Sekiya, C.; Namiki, M.; Fujimoto, Y.; Nagao, M. and Mori, M. **(1995):** Changes in free radical-metabolizing enzymes and lipid peroxides in the liver of Long-Evans with cinnamon-like coat color rats. *J. Gastroenterol.* **30,** 619-623.

Omaye, S. T.; Taylor, S. L.; Forstrom, J. W. and Tappel, A. L. **(1975):** Lipid peroxidation and reactions of glutathione peroxidase. *Fed. Proc.* **34,** 538 (Abst. 1797).

Palomero, J.; Galán, I.; Munoz, M. E.; Tunón, M. J.; González-Gallego, J. and Jiménez, R. **(2001):** Effect of aging on the susceptibility to the toxic effects of cyclosporin a in rats. Changes in liver glutathione and antioxidant enzymes. *Free Radic.Biol. Med.* **30,** 836-845.

Paniker, N. V.; Srivastava, S. K. and Beutler, E. **(1970):** Glutathione metabolism of the red cells. Effect of glutathione reducatase deficiency on the stimulation of hexose Monophosphate shunt under oxidative stress. *Biochim Biophys. Acta.* **215,** 456-460.

Paszkowski, T.; Traub, A. I.; Robinson, S. Y. and McMaster, D. **(1995):** Se dependent glutathione peroxidase activity in human follicular fluid. *Clin. Chim. Acta.* **236,** 173-180.

Powers, S. K.; Lawler, J.; Criswell, D.; Lieu, F.; Dodd, S. **(1992):** Alterations in diaphragmatic oxidative and antioxidant enzymes in the senescent Fisher 344 rats. *J. Appl. Phys.* **6,** 2317-2321.

Prohaska, J. R. and Ganther, H. E. **(1977):** Glutathione peroxidase activity of glutathione-S-transferases purified from rat liver. *Bioch. Biophs. Res. Commun.* **76,** 437-445.

Prohaska, J. R. and Sunde, R. A. **(1993):** Comparison of liver GSHPx activity and messenger-RNA in female and male mice and rats. *Comp. Biochem. Physiol*. **105B,** 111-116.

Ragusa, R. J.; Chow, C. K.; Clair, D. K. and Porter, J. D. **(1996):** Extraocular, limb and diaphragm muscle group-specific antioxidant enzyme activity patterns in control and mdx mice. *J. Neurol. Sci.* **139,** 180-186.

Rassaf, T.; Preik, M. and Kleinbongard, P. **(2002):** Evidence for *in vivo* transport of bioactive nitric oxide in human plasma. *J. Clin. Invest.* **109,** 1241-1248.

Reddy, K. and Tappel, A. L. **(1974):** Effect of dietary selenium and autoxidized lipids on the GSH system of gastrointestinal tract and other tissues in the rat. *J. Nutr*. **104,** 1069-1078.

Redmond, H. P.; Wang, J. H. and Bouchier-Hayes, D. **(1996):** Taurine attenuates nitric oxide- and reactive oxygen intermediate-dependent hepatocyte injury. *Arch. Surg.* **131,** 1280-1287.

Rocher, C.; Lalanne, J. L. and Chaudiere, J. **(1992):** Purification and properties of a recombinant sulfur analog of murine selenium-glutathione peroxidase. *Eur. J. Bioch.* **205,** 955-960.

Rotruck, J. T.; Hoekstra, W. G.; Pope, A. L.; Ganther, H. E.; Swanson, A. B. and Hafeman, D. G. **(1972):** Relationship of selenium to GSH peroxidase. *Fed. Proc.* **31,** 691.

Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G. and Hoekstra, W. G. **(1973):** Selenium: Biochemical role as a component of glutathione peroxidase. *Science.* **179,** 588-590.

Rusting, R. L. **(1992):** Trends in Biologx: Why do we age? *Scientific American,* December 130-141.

Saaranen, M.; Suistomaa, U. and Vanha-Perttula, T. **(1989):** Semen Se content and sperm mitochondrial volume in human and some animal species. *Human Reprod.* **4,** 304-308.

Sachdev, S. W., and Sunde, R. A. **(2001):** Selenium regulation of transcript abundance and translational efficiency of glutathione peroxidase-1 and -4 in rat liver. *Biochem. J.* **357,** 851-858.

Sagara, Y.; Dargusch, R.; Chambers, D.; Davis, J.; Schubert, D. and Maher, P. **(1998):** Cellular mechanisms of resistance to chronic oxidative stress. *Free Radic. Biol. Med.* **24,** 1375-1389.

Santa, M. C. and Machado, A. **(1986):** Effects of development and ageing on pulmonary NADPH-cytochrome C reductase, glutathione peroxidase, glutathione reductase and thioredoxin reductase activity in male and female rats. *Mech. Ageing Dev.* **37,** 183-195.

Sasaki, M.; Yoshida, M. C. and Kagami, K. **(1985):** Spontaneous hepatitis in an inbred strain of Long-Evans rats. *Rat Newslett.* **14,** 4-6.

Scarpa, M.; Rigo, A.; Viglino, P.; Stevanato, R.; Bracco, F. and Battistin, L. **(1987):** Age dependence of the level of the enzymes involved in the protection against active oxygen species in the rat brain. *Proc. Soc. Exp. Bio. Med.* **185,** 129-133.

Schenkman, J.; Frey, I.; Remmer, H. and Estabrook, R. **(1967):** *Mol. Pharmacol.* **3,** 516-525.

Schisler, N. J. and Singh, S. M. **(1988):** Modulating of Se- GSHPx activity in mice. *Free Radic. Biol. Med.* **4,** 147-153.

Schuckelt, R.; Brigeliusflohe, R.; Maiorino, M.; Roveri, A.; Reumkens, J. and Strassburger, W. **(1991):** Phospholipid hydroperoxide glutathione peroxidase is a seleno-enzyme distinct from the classical glutathione peroxidase as evident from cDNA and amino acid sequencing. *Free Rad. Res. Comm.* **14,** 343-361.

Sedlak, J. and Lindsay, R. H. C. **(1968):** Estimation of total, protein-bound and non-protein sulfhydryl groups in tissue with Ellmann's reagent. *Anal. Biochem.* 25, 192-205.

Shen, Y.; Engberg, R. and Jakobsen, K. **(1992):** On the requirement of vitamin E in fast and slow growing chickens experiments with broiler and leghorn type chickens. *J. Anim. Phys. Anim. Nutr.* **67,** 113-122.

Sies, H. **(1986):** Biochemistry of oxidative stress. *Chem. Int. Ed. Engl.* **25,** 1058-1071.

Sies, H. **(1999):** Glutathione and its role in cellular functions. *Free Radic. Biol. Med.* **27,** 916.

Sies, H. and Moss, K. M. **(1978):** A role of mitochondrial GSHPx in imodulating-mitochondrial oxidation in liver. *Eur. J. Biochem.* **84,** 377-383.

Sies, H.; Gerstenecker, C.; Summer, K. H.; Menzel, H. and Flohé, L. **(1974):** GSHPx metabolism and associated metabolic transitions in hemoglobin-free perfused rat liver. In: Flohé, L., Benöhr, H. Ch., Sies H., Waller, H. D. and Wendel, A., eds.: Glutathione, pp. 261-267, Georg Thieme, Stuttgart.

Sies, H.; Gertenecker, C.; Menzel, H. and Flohé, L. **(1972):** Oxidation in the NADP system and release of GSSG from hemoglobin-free perfused rat liver during peroxidatic oxidation of glutathione by hydroperoxides. *FEBS Lett.* **27,** 171-175.

Smith, J. and Shrift, A. **(1978):** Phylogenetic distribution of glutathione peroxidase. *Comp. Bioc. Physiol.* **63B.** 39-44.

Sohal, R. S. and Weindruch, R. **(1996):** Oxidative stress, caloric restriction, and aging. *Science* **272,** 1010-1013.

SPSS For WINDOWS **(1999):** Version 9, Copyright SPSS Inc.

Squires, E. J. and Wu, J. **(1992):** Enhanced induction of hepatic lipid peroxidation by ferric nitrilotriacetate in chickens susceptible to fatty liver rupture. *Br. Poult. Sci.* **33,** 329-337.

Stadman, E. R. **(1981):** Protein oxidation and aging. *Science.* **257,** 1220-1224.

Stahl, E. **(1958):** Dünnschicht-chromatographie. II. standardisierung, sichtbarmachung dokumentation and anwendung. *Chem. Ztg.* **82,** 341-329.

Stowe, H. D. and Miller, E. R. **(1985):** Genetic predisposition of pigs to hypo- and hyperselenemia. *J. Anim. Sci.* **60,** 200-211.

Sunde, R. A. **(1980):** Selenium. In: O'Dell, B. L. and Sunde, R. A. eds.: *Handbook of Nuritionally Essential Mineral Elements*, Vol. 18, pp. 493-556, Marcel Dekker, New York.

Sunde, R. A. **(1990):** Molecular biology of selenoproteins. *Ann. Rev. Nutr.* **10,** 451-474.

Sunde, R. A., Dyer, J. A., Moran, T. V., Evenson, J. K. and Sugimoto, M. **(1993):** Phospholipid hydroperoxide glutathione peroxidase: full-length pig blastocyst cDNA squence and regulation by selenium status. *Bioch. Biophys. Res. Commun.* **193,** 905-911.

Surai, P. F. **(1999a):** Tissue-specific changes in the activity of antioxidant enzymes during the development of the chicken embryo. *Br. Poult. Sci.* 40, 397-405.

Surai, P. F. **(1999b):** Vitamin E in avian reproduction. *Poult. Avian Biol. Rev.* **10,** 1-60

Surai, P. F. **(2000):** Organic Se: benefits to animals and humans, a biochemist's view. In: Lyons, T. P. and Jaques, K. A. eds.: Biotechnology in feed industry. Proc. Alltech's 16th Annual Symposium, pp. 205-260, Nottingham University Press, Nottingham.

Surai, P. F. **(2002):** Natural antioxidants in avian nutrition and reproduction. Nottingham University Press.

Surai, P. F. and Dvorska, J. E. **(2001):** Is organic selenium better for animals than inorganic sources? Two different scenarios in stress conditions *Feed Mix.* **9. 4**/5: 8-10.

Surai, P. F. and Sparks, N. H. C. **(2001):** Designer eggs: from improvement of egg composition to functional food. *Trends in Food Sci. and Technology.* **12,** 7-16.

Surai, P. F., Brillard, J. P., Speake, B. K., Blesbois, E., Seigneurin, F. Sparks, N. H. C. **(2000):** Phospholipid fatty acid composition, vitamin E content and susceptibility to lipid peroxidation of duck semen. *Theriogenology.* **53,** 1025-1039.

Surai, P. F., Cerolini, S., Wishart, G., Speake, B. K., Noble, R. C., and Sparks, N. H. C. **(1998b):** Lipid and antioxidant composition of chicken semen and its susceptibility to peroxidation. *Poult. Avian. Biol. Rev.* **9,** 11-23.

Surai, P. F., Kostjuk, I., Wishart, G., Macpherson, A., Speake, B., Noble, R., Ionov, I. and Kutz, E. **(1998c):** Effect of vitamin E and selenium of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes and liver. *Biol.Trace Elem. Res.* **64,** 119-132.

Surai, P. F.; Blesbois, E.; Grasseau, I.; Ghalah, T.; Brillard, J. P.; Wishart, G.; Cerolini, S. and Sparks, N. H. C. **(1998a):** Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. *Comp. Biochem. Physiol.* **120B,** 527-533.

Syväjärvi, J.; Saloniemi, H. and Gröhn, Y. **(1986):** An epidemiological and genetic study on registered diseases in Finnish Ayreshire cattle. IV. Clinical mastitis. *Acta. Vet. Scand.* **27,** 223-234.

Takahashi, K. and Cohen, H. J. **(1986):** Selenium-dependent glutathione peroxidase protein and activity: immunological investigations on cellular and plasma enzymes. *Blood.* **68,** 640-645.

Takahashi, K.; Avissar, N.; Whitin, J. and Cohen, H. **(1987):** Purification and characterisation of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the cellular enzyme. *Arch. Biochem. Biophys.* **256,** 677-686.

Takahashi, K.; Akasaka, M.; Yamamoto, Y.; Kobayashi, C.; Mizoguchi, J. and Koyama, J. **(1990):** Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences. *J. Biochem.* **108,** 145-148.

Tam, B. K. and McCay, P. B. **(1970):** Reduced triphosphopyridine nucleotide oxidase-Catalyzed alterations of membrane phospholipids. *J. Biol. Chem.* **245,** 2295-2300.

Taniguchi, M. **(1997):** Effect of N-nitrosodimethylamine on glutathione levels during development of chick-embryo. *Chem.-Biol.Interactions.* **105,** 65-75.

Tappel, M. E.; Chaudier, J. and Tappel, A. L. **(1982):** GSHPx activity in animal tissues. *Comp. Biochem. Physiol.* **4,** 945-949.

Tham, D. M., Whitin, J. C., Kim, K. K., Zhu, S. X. and Cohen, H. J. **(1998):** Expression of extracellular glutathione peroxidase in human and mouse gastrointestinal tract. *Am. J. Physiol. Gastrointest.* **38,** G1463-G1471.

Toren, F. and Nikki, J. **(2000):** Oxidants, Oxidative stress and the biology of aging. *Nature.* **408,** 239-247.

Toshinai, K.; Ohishi, S.; Kizaki, T.; Ookawara, T.; Haga, S. and Ohno, H. **(1997):** Effect of swimming training on antioxidant enzymes in kidney of zoung and old mice. *Res. Commun. Mol. Pathol. Pharmaco.* **95,** 3-12.

Toyoda, H.; Himeno, S. and Imurz, N. **(1989):** The regulation of GSHPx gene expression relevant to species difference and the effects of dietary Se manipulation. *Biochim. Biophys. Acta.* **1008,** 301-308.

Tsan, M. F. **(1997):** Superoxide dismutase and pulmonary oxygen toxicity. *Proc. Soc. Exp. Biol. Med.* 214, 107-113.

Tucker, E. M., Kilgour, L. **(1970):** *Comp.Biochem.Physiol.* **46,** 93-107.

Ursini, F.; Maiorino, M. and Gregolin, C. **(1985):** The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim. Biophys. Acta.* **839,** 62-70.

Ursini, F.; Majorino, M.; Flohe, R.; Aumann, K. D.; Roveri, A. and Schomburg, D. **(1995):** Diversity of glutathione peroxidase. Biothiols, Part B. *Meth .Enzymol.* **252,** 38-53.

Van Lieshout, E. M. and Peters, W. H., **(1998):** Age and gender dependent levels of glutathione and glutathione-S-transferase in human lumphocytes. *Carcinogenesis.* **10,** 1873-1875.

Vericel, E.; Narce, M.; Ulmann, L.; Poisson, J. P. and Lagarde, M. **(1994):** Age-related changes in antioxidant defence mechanisms and peroxidation in isolated hepatocytes from spontaneously hypertensive and normotensive razs. *Mol. Cell. Biochem.* **132,** 25-29.

Vernet, P.; Rigaudiere, N.; Ghyselinck, N.; Dufaure, J. P. and Drevet, J. R. **(1996):** *In vitro* expression of a mouse tissue specific glutathione-peroxidase like protein laking the selenocysteine can protect stably transfected mammalian cells against oxidative damage. *Biochem. Cell. Biol.* **74,** 125-131.

Vertechy, M.; Cooper, M.; Ghirardi, O. and Ramacci, M. **(1993):** The effect of age on the activity of enzymes of peroxide metabolism in rat brain. *Exp. Gerontol.* **1,** 77-85.

Virág, Gy.; Mézes, M.; Szendrõ, Zs.; Romvári, R.; Radnai, I. and Bíróné, E. **(1996):** Moderate phenotypic relationships between glutathione peroxidase activity and carcass traits in rabbit could be partially determined by genetic effect, Proc. 6th World Rabbit Congr. Toulouse, Vol. 2, 381-384.

Vleck, C. M. and Hoyt, D. F. **(1991):** Metabolism and energetics of reptillian and avian embryos, In: Deeming, C. and Ferguson, M. eds.: Egg incubation: its effects on embryonic development in birds and reptiles. Cambridge University Press, Cambridge, pp. 258-306.

Vohra, B. P.; Sharma, S. P. and Kansal, V. K. **(2001):** Effect of Maharishi Amrit Kalash on age dependent variations in mitochondrial antioxidant enzymes, lipid peroxidation and mitochondrial population in different regions of the central nervous system of guinea-pigs. *Drug Metabol Drug Interact.* **18,** 57-68.

Wang, S. T.; Chen, H. W.; Sheen, L. Y. and Lii, C. K. **(1997):** Methionine and cysteine affect glutathione level, glutathione-related enzyme activity and the expression of glutathione S-transferase isozymes in rat hepatocytes. *J. Nutr.* **127,** 2135-2141.

Weichselbaum, T. E. **(1946):** An accurate and rapid method for the determination of protein in small amounts of serum and plasma. *Am. J. Clin. Pathol.* **16,** 40-43.

Weiss, S. L.; Evenson, J. K.; Thompson, K. M. and Sunde, R. A. **(1997):** Dietary selenium regulation of glutathione peroxidase mRNA and other selenium-dependent parameters in male rats. *J. Nutr. Biochem.* 8, 85-91.

Weiss, W. P. **(1998):** Requirements of fat-soluble vitamins for dairy cows: A review. *J. Dairy Sci.* **81,** 2493-2501.

Westermarck, T. **(1977):** Selenium content of tissues in finnish infants and adults with various diseases, and studies on the effects of selenium supplementation in neuronal ceroid lipofuscinosis patients. *Acta Pharmacol. Toxicol*. **41,** 121-128.

Wendel, A.; Pilz, W.; Ladenstein, R. Sawatzki, G. and Weser, U. **(1975):** Substrate-induced redox change of selenium in glutathione peroxidase studied by x-ray photo-electron Spectroscopy. *Biochim Biophys. Acta.* **377,** 211-215.

Wiener, G.; Woolliams, J. A. and Vagg, M. G. **(1983):** Selenium concentration in the blood and wool and glutathione peroxidase activity in the blood of three breeds of sheep. *Res. Vet. Sci.* **34,** 265-266.

Williams, J. G.; Kubelik, R. A.; Livak, K. J.; Rafalski J. A. and Tingey, S. V. **(1990):** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* **18,** 6531.

Williams, K., Frayne, J. and Hall, L. **(1998):** Expression of extracellular glutathione peroxidase type 5 (GPX-5) in the rat male reproductive tract. *Mol. Hum. Reprod.* **4,** 841-848.

Wilson, J. X.; Lui, E. M. K. and Del Maestro, R. F. **(1992):** Development profiles of antioxidant enzymes and trace metals in chick embryo. *Mech. Aging Dev.* **65,** 51-64.

Winkler, J. **(1921):** Poultry breeding. Pátria, Budapest (in Hungarian).

Woolliams, J. A.; Wiener, G.; Anderson, P. H.; Mcmurray, C. H. **(1983):** Variation in the activity of glutathione peroxidase and superoxide dismutase and the concentration of copper in the blood in various breed crosses of sheep. *Res. Vet. Sci.* **34,** 253-256.

Wu, G. and Meininger, C. J. **(2000):** Arginine nutrition and cardiovascular function. *J. Nutr.* **130,** 2626-2629.

Wu, G. and Morris, S. M. **(1998):** Arginine metabolism: nitric oxide and beyond. *Biochem J.* 336, 1-17.

Wu, G.; Flynn, N. E.; Flynn, S. P.; Jolly, C. A. and Davis, P. K. **(1999):** Dietary proteion or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. *J. Nutr.* **129,** 1347.

Wu, J. and Squires, J. **(1997):** The effect of dietary corn oil, vitamin E, and Se on lipid peroxidation and hemorrhage in chicken liver. *J. Nutr. Biochem.* **8,** 629-633.

Yidiz, S.; Naziroglu, M.; Kaya, I.; Aydilek, N. and Yuce, A. **(2002):** Effects of palm oil on lipid peroxidation, reduced glutathione, glutathione peroxidase, and vitamin A levels in the Corpus uteri, Cornu uteri and Corpus Iuteum of young and adult female sheep. *J Vet. Med. A* **49,** 373-378.

Yu, B. P. **(1994):** Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.* **74,** 139-162.

Yuan, H.; Kaneko, T.; Kaji, K.; Kondo, H. and Matsuo, M. **(1995):** Species difference in the resistibility of embryonic fibroblasts against oxygen-induced growth inhibition. *Comp Biochem Physiol* **110B,** 145-154.

Zheng, M. and Storz, G. **(2000):** Redox sensing by prokaryotic transcription factors. *Biochem Pharmacol.* **59,** 1-6.

11. ACKNOWLEDGEMENTS

First and foremost, all praises and limitless thanks are devoted to God who gave me the capability to do this study.

I wish to express my deepest thanks, gratitude and sincerest appreciation to my co-advisors, Dr. András Hidas, senior research scientist, Department of Breeding and Genetics, Institute for Small Animal Research, Gödöllõ, Hungary, and Prof. Miklós Mézes, Professor of Nutrition, Department of Nutrition, Szent István University, Faculty of Agricultural and Environmental Sciences, Gödöllő, Hungary, for suggesting the problem and supervising this work. Their exceptional supervision, understanding, continuous patience and encouragement, valuable advice and guidance during this investigation, as well as warm friendships are highly acknowledged.

I would like to thank Dr. Márta Erdélyi, Department of Nutrition, Szent István University, Faculty of Agricultural and Environmental Sciences, Gödöllő, Hungary, for her helpful and excellent advice.

The excellent technical assistance of Ms. Erika M. Edvi and Ms. Ilona L. Lipcsei, Department of Breeding and Genetics, Institute for Small Animal Research, Gödöllõ, Hungary and Ms. Mónika Varsányi and Krisztián Balogh, Department of Nutrition, Szent István University, Faculty of Agricultural and Environmental Sciences, Gödöllő, is also acknowledged.

Deep thanks and sincere appreciation due the Department of Breeding and Genetics, Institute for Small Animal Research, Gödöllõ, Hungary, for their kindness and help and making my work pleasant and enjoyable.

Deep thanks due to Dr. István Szalay and Ms. Ildikó Barta, Department of Gene Conservation, Institute for Small Animal Research, Gödöllő, Hungary, for providing the breeds used in this study.

I specially want to thank my parents, my brothers and my sisters, who have always supported my heart and soul with endless love and encouragement. Without them, I could-not go so far towards my professional goals. Last but not least, special thanks are extended to my husband Dr. Eissa Ahmed Eissa and my sons, Ahmed, Eslam and Mohamed who have always supported my heart and soul with endless love and encouragement.

12. APPENDIX

Hungarian White Male

Hungarian White Female

Hungarian Speckled Male

Hungarian Speckled female

Transylvanian Naked Neck Black Male

Transylvanian Naked Neck Black Female

Transylvanian Naked Neck White Male

Transylvanian Naked Neck White Female

New Hampshire Male

New Hampshire Female