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E-mail: editorijps@gmail.com

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Comparative Effects of Various Forms of Selenium on Thioredoxin Reductase Activity in Broiler Chickens

K.M. Gowdy[1], F.W. Edens[1] and K.Z. Mahmoud[2]
[1]Department of Poultry Science, North Carolina State University, Raleigh, NC 27695, USA
[2]Department of Animal Production, Jordan University of Science and Technology, Irbid 22110, Jordan

Abstract: An experiment was conducted to measure thioredoxin reductase (TrxR) and glutathione peroxidase (GSH-Px) enzymatic activity in organs and TrxR activity in liver subcellular fractions in young broiler chickens. Broilers were fed either a (1) Control basal diet (no supplemental selenium but with a background level of 0.095 mg/kg) or diets providing supplemental selenium at 0.3 mg/kg as either (2) sodium selenite (SE; inorganic selenium), (3) Sel-Plex® (SY; organic selenium yeast), or (4) a combination (SS) of 0.15 mg/kg of both selenium forms. GSH-Px (measured in liver only) and TrxR activities were elevated by selenium, regardless of dietary form, in each organ examined. The TrxR activities in subcellular fractions were greatest in the mitochondrial lysate, nuclear pellet and post-mitochondrial supernatant, respectively and the lowest activity was associated with the mitochondrial pellet. Aurothioglucose (ATG; 0.1 μM/g BW) inhibited hepatic cell GSH-Px activity by more than 30% and TrxR activity by more than 80%, but glutathione reductase was not affected. The TrxR enzyme in the chicken might be different from the mammalian enzyme.

Key words: Chickens, selenium, thioredoxin reductase

INTRODUCTION

Selenium is involved in numerous physiological functions with its most notable role as the critical factor activating members of the selenoprotein family. The most abundant selenoproteins in mammals are glutathione peroxidase (GSH-Px; EC 1.11.1.9) and thioredoxin reductase (TrxR; EC 1.8.1.9) (Gladyshev *et al*., 1998; Rotruck *et al*., 1973). The expression of TrxR in mammals is dependent on the amount of available selenium, various hormones and environmental conditions, and oxidative stressors (Berggren *et al*., 1997, 1999; Kohrl *et al*., 2000). TrxR is a widely distributed flavoprotein that catalyzes the crucial NADPH-dependent reduction of thioredoxin protecting cellular components from oxidative damage (Ganther, 1999) and through the reduction of thioredoxin, the thioredoxin-TrxR system catalyzes the reduction of oxidized proteins and many other substrates such as lipid peroxides, thyroid peroxidase, glutathione, ascorbate, selenite, selenodiglutathione and protein disulfide isomerase (Chae *et al*., 1994; Luthman and Holmgren, 1982).

An extensive examination of TrxR in chickens has not been conducted. Burgos *et al*. (2006) reported that reovirus infection had no influence on TrxR activity in neither the intestinal ileum nor the liver and Burgos *et al*. (2006) noted that both inorganic and organic selenium dietary supplements increased TrxR activity in both ileum and liver compared with the same tissues in young broilers fed a selenium deficient diet (Se<0.02 mg/kg). These observations suggested that TrxR in chickens might respond similarly to TrxR in mammals that had been fed either selenium-deficient or selenium-adequate diets (Berggren *et al*., 1997, 1999; Barnes *et al*., 2009; Gallegos *et al*., 1997; Hill *et al*., 1997; Humann-Ziehank *et al*., 2013).

The study of Upton *et al*. (2008) demonstrated that the form of dietary selenium can alter the activity of GSH-Px responding to oxidative stress resulting from higher rates of metabolism in high-yielding broiler chickens and oxidation of feed ingredients such as dietary fat. Since oxidative stress can be induced by numerous factors in poultry species and partially ameliorated by the provision of organic selenium (Mahmoud and Edens, 2003; Upton *et al*., 2008), it was important to ascertain whether dietary inorganic and organic selenium might affect differentially TrxR activity in chickens. The objective of this study was to use different chemical forms of selenium (inorganic sodium selenite vs. organic selenium as a selenium yeast product (SY), Sel-Plex®) in broiler diets and assess their influence on subcellular and organ distribution of TrxR activity.

MATERIALS AND METHODS

Broiler chickens and husbandry: This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

Ross male broilers were fed the North Carolina State University's North Carolina Agricultural Research Service broiler starter feed (Table 1), which provided

Corresponding Author: Frank W. Edens, Department of Poultry Science, North Carolina State University, Box 7635 Raleigh, NC 27695

Table 1: Composition (g/kg) of North Carolina Agricultural Research Service broiler starter diets supplemented with either no selenium (Control), inorganic sodium selenite (SE), organic selenium yeast (SY), or a combination of SE and SY

Ingredients	Control[3]	SE[3]	SY[3]	SS[3]
Corn	590.8	590.8	590.8	590.8
Soy	269.5	269.5	269.5	269.5
Limestone	7.0	7.0	7.0	7.0
Dical phosphate	7.0	7.0	7.0	7.0
Poultry fat	34.9	34.9	34.9	34.9
Poultry meal	79.8	79.8	79.8	79.8
DL-methionine	1.8	1.8	1.8	1.8
Lysine	0.7	0.7	0.7	0.7
Salt	4.0	4.0	4.0	4.0
Choline chloride	2.0	2.0	2.0	2.0
Minerals[1] (TM-90)	2.0	2.0	2.0	2.0
Vitamins[2] (NCSU-90)	0.5	0.5	0.5	0.5
Sodium Selenite	0.0	1.0	0.0	0.5
Sel-Plex®	0.0	0.0	1.0	0.5

[1]Trace mineral (TM-90) premix provided in mg/kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1.0 mg. Selenium premix as either sodium selenite or organic selenium (Sel-Plex®) was provided to each diet at a level to assure a maximum concentration of 0.3 mg/kg

[2]Vitamin premix (NCSU-90) provided per kilogram of diet: Vitamin A (retinyl acetate), 2.27 mg; Vitamin B_1 (thiamine), 2 mg; Vitamin B_2 (riboflavin), 6.6 mg; Vitamin B_3 (niacin), 55 mg; Vitamin B_5 (pantothenic acid), 11 mg; Vitamin B_8 (pyridoxine), 4 mg; Vitamin B_7 (biotin), 1.26 mg; Vitamin B_9 (folic acid), 1.1 mg; Vitamin B_{12} (cyanocobalamin), 0.0198 mg; Vitamin D_3 (cholecalciferol), 0.05 mg; Vitamin E (DL-alpha-tocopheryl acetate), 33 mg; Vitamin K_3 (menadione), 2 mg

[3]Control-no supplemental selenium; SE-inorganic sodium selenite at 0.3 mg/kg; SY-organic selenium yeast at 0.3 mg/kg; SS-SE at 0.15 mg/mg+SY at 0.15 mg/kg

13.21 Mj/kg metabolizable energy (ME) and 21% crude protein (CP) from 1d of age to 3 wk of age. A basal feed (Control) with a background level of 0.095 mg/kg selenium (North Carolina Department of Agriculture, Raleigh, North Carolina 27607) was supplemented with either 0.3 mg/kg of sodium selenite (SE), Sel-Plex® (a selenium yeast protein (SY) produced and marketed by Alltech, Inc., Nicholasville, Kentucky), or a combination 0.15 mg/kg of both SE and SY (SS).

The study was conducted in 2 battery trials with 3 replicates per treatment using 10 birds per replicate. Replicates were arranged in a completely randomized design with blocking for light and position within the brooder battery. Birds were given feed and water for *ad libitum* consumption. Broilers used for tissue sampling and enzyme analysis were removed randomly from the various dietary selenium treatment pens.

Activities of TrxR in various organs: At 3 weeks of age, liver and other tissue samples (0.5-1.0 g; lung, heart, kidney, brain, breast muscle, bursa of Fabricius, thymus, spleen, red blood cells and plasma) were placed directly into ice-cold protein sample buffer (50 mM potassium phosphate buffer, pH 7.4, 1 mM phenylmethyl sulfonyl fluoride and 5 mM ethylenediaminetetraacetic acid (EDTA). All chemicals, unless noted otherwise, were purchased from Sigma-Aldrich (St. Louis, Missouri).

Each tissue sample was washed to rinse away adhering blood and samples were then minced in 4 mL of cold protein sample buffer for protein extraction. The samples were homogenized with a Polytron Homogenizer (Heat System Ultrasonics, Plainview, N.Y. 11803) in cold 50 mM TrisHCl, 1mM EDTA pH 7.5 in a 1:3 ratio. The resulting homogenates were centrifuged at 6,500 x g for 30 min and supernatants were removed and further centrifuged at 50,000 x g for 30 min. Supernatants were removed and analyzed for TrxR activity using the 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) assay described by Luthman and Holmgren (1982). Working buffer (100 mM sodium phosphate, 10 mM Na_4EDTA, 0.2 mM NADPH, 0.2 mg bovine serum albumin (BSA)/mL, 1% ethanol, 5 mM DTNB) and 0.5 mM flavin adenine dinucleotide (FAD) (4.2 mg of FAD in 500 mM Tris, pH 7.4) was added to a 96 well plate before adding sample. Absorbency was read at 412 nm for 3 min. Samples were run in triplicate and results were calculated based on the yield of 2 moles of 2-nitro-5-thiobenzoate per mol of NADPH consumed (nmol /min/mg protein).

The GSH-Px activity in liver cytosolic protein extract was determined by a spectrophotometric method (St. Clair and Chow, 1996) based on the decrease in β-Nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt (NADPH) absorbance at 340 nm. The rate of absorbance decrease at 340 nm is directly proportional to the GSH-Px activity (U/mg total protein). Hepatic glutathione reductase (GR) activity (U/mg total protein) in liver cytosolic protein extract was determined spectrophotometrically based on the measurement of the rate of NADPH oxidation at 340 nm with synchronized reduction of glutathione disulfide (GSSG) by using a commercial kit system (Oxis International, 2009).

Subcellular distribution of TrxR activity in liver cells: Livers from five 3 weeks old Ross male broilers, fed SY at 0.3 mg/kg of diet, were collected fresh, minced and washed immediately in ice cold 0.9% saline. The minced liver was then diluted to a 1:5 (w:v) mixture with an ice cold 250 mM sucrose, 5 mM Tris HCl pH 7.5 buffer. Tissue was ground with a 40 mL Dounce homogenizer with a glass pestle. The homogenate was spun at 1,500 x g for 30 min at 4°C followed by removal of the post-nuclear supernatant and isolation of the nuclear pellets. The post-nuclear supernatant was centrifuged at 12,000 x g for 30 min at 4°C, which yielded the mitochondrial pellet and post-mitochondrial supernatant. The mitochondrial pellets were resuspended in 5 mM Tris HCl pH 7.5 and homogenized with the Dounce homogenizer. The pooled pellets were then centrifuged at 12,000 x g for 30 min at 4°C, which yielded the mitochondrial lysate and mitochondrial membranes. A 2 mL aliquot of each fraction was taken and analyzed for protein and TrxR activity.

Total protein determination: Total protein content was measured spectrophotometrically at 595 nm using the BioRad Bradford Protein Assay Kit (Hercules, CA 94547) modified for a microplate reader and used BSA (Sigma, St. Louis, MO) as a standard. The standard curve was linear ($r^2 \geq 0.98$) from 0 to 80 µg/mL of BSA. Coefficients of variation, between replicates of the same sample, were less than 5%.

Administration of aurothioglucose: The effects of aurothioglucose (ATG; Sigma Chemical Co., St. Louis, MO) on hepatic TrxR activity was examined in three weeks old broiler chicks from each Control, SE, SY and SS feed treatment group. Forty chicks were involved in this experiment. There were 10 chicks per group (Control, SE, SY and SS) of which five per group served as the 0 h controls and five per group were treated with ATG dissolved in sterile saline and injected intra-abdominally with 0.1 µM ATG/g BW. The 0 h broilers from each dietary treatment group were euthanized and followed immediately by dissection and collection of approximately 1.0 g of liver tissue. At 6 hours post-injection, ATG-treated chickens were euthanized and followed immediately by dissection of approximately 1.0 g liver tissue. The liver tissues were then processed as described above for collection of the post-mitochondrial supernatant fraction, which was assayed for GSH-Px, GR and TrxR activity as described above.

Data analysis: Data from all experiments were analyzed using the ANOVA procedure of the Statistical Analysis System (SAS Institute, 2004). Differences among means were determined with the Student Newman Kuehls test. The level of significance was set at $p<0.05$.

RESULTS

The GSH-Px, GR and TrxR activities in the liver of the broiler chickens fed either Control (no supplemental selenium), SE, SY, or SS are shown in Table 2. The activities of liver cytosolic GSH-Px and TrxR in broilers fed supplemental dietary selenium as SE, SY and SS were elevated significantly ($p \leq 0.05$) and equivalently compared to GSH-Px and TrxR in broilers fed the Control diet. The GR activity was not altered by supplemental dietary selenium treatments (Table 2).

The TrxR activities in the liver, heart, breast muscle, bursa of Fabricius, thymus and spleen are selenium dependent (Table 3). The Se-deficient control chickens had the lowest TrxR activity in the majority of the organs (Table 3). TrxR activity in the heart, breast muscle, bursa of Fabricius, spleen and thymus were found to be numerically greater in SY-fed birds in most instances, but not significantly different from the other selenium treatment groups (Table 3). TrxR activity in lung, kidney, brain was elevated, but not significantly, by supplemental selenium treatments and lack of a significant treatment effect was attributed to the high variability of the TrxR activities found in these tissues. TrxR activities in red blood cells and plasma were highly variable and were not affected significantly by the selenium diets (Table 3).

The subcellular distribution of TrxR activity in hepatic cells from chickens fed SY is presented in Table 4. The TrxR activity of the liver homogenate was 107.2 nmol/min/mg protein, which is the composite of all the subcellular TrxR activities. Among the subcellular fractions, the highest TrxR activities were measured in the mitochondrial lysate (133.3 nmol/min/mg protein), nuclear pellet (128.9 nmol/min/mg protein) and post-mitochondrial supernatant (106.6 nmol/min/mg protein),

Table 2: Activities of liver glutathione peroxidase (GSH-Px), glutathione reductase (GR) and thioredoxin reductase (TrxR) in three weeks old male broiler chickens fed diets differing in selenium sources

Enzymes	Control[4]	SE[4]	SY[4]	SS[4]	Pooled SEM
GSH-Px[1]	0.193[b]	0.319[a]	0.343[a]	0.332[a]	0.038
GR[2]	0.219[a]	0.227[a]	0.226[a]	0.218[a]	0.046
TrxR[3]	22.3[b]	65.4[a]	70.2[a]	68.4[a]	15.7

[a,b]In a row, means with unlike superscripts differ significantly ($p \leq 0.05$)
[1]Glutathione peroxidase (GSH-Px, U/min/mg protein)
[2]Glutathione reductase (GR, U/min/mg protein),
[3]Thioredoxin reductase (TrxR, nmol/min/mg protein)
[4]Control-no supplemental selenium; SE-inorganic sodium selenite at 0.3 mg/kg; SY-organic selenium yeast at 0.3 mg/kg; SS-SE at 0.15 mg/mg+SY at 0.15 mg/kg

Table 3: Thioredoxin reductase (TrxR) activity (nmol/min/mg protein) in organs from three weeks old male broiler chickens fed diets differing in selenium sources

Tissues	Control[1]	SE[1]	SY[1]	SS[1]
Liver	20.4±10[b]	79.9±39[a]	78.1±51[a]	51.0±20[ab]
Lung	51.2±50[a]	81.1±31[a]	91.1±69[a]	79.0±11[a]
Heart	21.0±11[b]	62.1±29[b]	109.9±41[a]	59.9±21[b]
Kidney	62.0±69[a]	89.9±10[a]	81.0±31[a]	51.0±31[a]
Brain	51.1±41[a]	85.4±32[a]	152.0±99[a]	133.2±101[a]
Breast muscle	30.9±12[b]	72.2±19[ab]	79.9±39[a]	70.9±29[ab]
Bursa	29.9±11[b]	71.9±19[ab]	100.0±49[a]	60.4±29[ab]
Thymus	40.5±21[c]	90.8±29[b]	139.9±39[a]	80.7±31[b]
Spleen	32.0±9[b]	41.1±10[ab]	71.0±39[a]	79.9±19[a]
RBC	30.9±31[a]	70.8±61[a]	40.4±20[a]	22.3±11[a]
Plasma	31.1±9[a]	30.9±10[a]	30.5±35[a]	39.8±19[a]

[a,b,c]In a row, means with unlike superscripts differ significantly ($p \leq 0.05$).
[1]Control-no supplemental selenium; SE-inorganic sodium selenite at 0.3 mg/kg; SY-organic selenium yeast at 0.3 mg/kg; SS-SE at 0.15 mg/mg+SY at 0.15 mg/kg

Table 4: Subcellular distribution of chicken thioredoxin reductase (TrxR) activity (nmol/min/mg protein) in liver cells from broiler chickens fed organic selenium yeast at 0.3 mg/kg feed

Subcellular distribution	Activity nmol/min/mg Protein[1]	Subcellular percentage TrxR Activities vs. Liver Homogenate
Liver Homogenate	107±9.1[a]	100.00
Nuclear Pellet	129±9.7[a]	120.10
Post Nuclear Supernatant	87±10.2[ab]	81.31
Mitochondria Pellet	65±8.3[b]	60.74
Post Mitochondria Supernatant	107±11.3[a]	100.00
Mitochondria Lysate	133±12.5[a]	124.30
Mitochondria Membranes	93±13.0[ab]	86.92

[a,b]In a column, means±SEM with unlike superscripts differ significantly ($p \leq 0.05$). [1]N = 3 for each mean±SEM

Table 5: Influence of aurothioglucose (0.1 μM/g BW) injection on hepatic glutathione peroxidase (GSH-Px), glutathione reductase (GR) and thioredoxin reductase (TrxR) activities in three weeks old male broilers fed diets differing in selenium sources

Enzymes	Control[4]	SE[4]	SY[4]	SS[4]
GSH-Px[1]				
0 h[Z]	0.122±0.014[a,Y]	0.160±0.006[a,XY]	0.203±0.014[a,X]	0.196±0.019[a,X]
6 h[Z]	0.081±0.008[b,Y]	0.108±0.002[b,XY]	0.138±0.019[b,X]	0.131±0.015[b,X]
% change	-33.6	-32.3	-32.0	-33.2
GR[2]				
0 hours[Z]	0.126±0.012	0.131±0.011	0.129±0.016	0.124±0.014
6 hours[Z]	0.123±0.011	0.132±0.017	0.130±0.019	0.125±0.016
% change	-2.4	+0.8	+0.8	+0.8
TrxR[3]				
0 h[Z]	31.2±8.1[a,Y]	101.9±9.2[a,X]	114.7±9.9[a,X]	104.3±10.6[a,X]
6 h[Z]	4.2±1.1[b,Y]	16.3±2.3[b,X]	21.6±2.1[b,X]	16.9±1.6[b,X]
% change	-86.54	-84.00	-81.17	-83.80

[a,b]In a column, enzyme activity means with unlike lower case superscripts differ significantly ($p \leq 0.05$)

[X,Y]In a row, enzyme activity means followed by unlike upper case superscripts differ significantly ($p \leq 0.05$)

[Z]N = 5 for each mean±SEM

[1]Glutathione peroxidase (GSH-Px, U/min/mg protein)

[2]Glutathione reductase (GR, U/min/mg protein),

[3]Thioredoxin reductase (TrxR, nmol/min/mg protein)

[4]Control-no supplemental selenium; SE-inorganic sodium selenite at 0.3 mg/kg; SY-organic selenium yeast at 0.3 mg/kg; SS-SE at 0.15 mg/kg+SY at 0.15 mg/kg

respectively. The lowest TrxR activity in the subcellular fractions was found in the mitochondrial pellet (65.0 nmol/min/mg protein), which was significantly lower ($p \leq 0.05$) than TrxR activities found in the nuclear pellet, post-mitochondrial supernatant and mitochondrial lysate but not different ($p \leq 0.05$) from TrxR activities found in post-nuclear supernatant and mitochondrial membranes. Contribution of these subcellular TrxR activities as a percentage of total liver homogenate activity is also shown in Table 4. The percentage TrxR activities in the nuclear pellet (120.24% of the total) and the mitochondrial lysate (124.35% of the total) were the highest followed by post-mitochondrial supernatant (99.44% of the total), mitochondrial membranes (86.75% of the total), post-nuclear supernatant (80.69% of the total) and the mitochondrial pellet (60.63%), respectively.

The results of ATG inhibition of GSH-Px and TrxR activities in the liver post-mitochondrial supernatant are shown in Table 5. There was no interaction between selenium forms and ATG treatment. Again, supplementation of selenium in both forms and their combination caused both GSH-Px and TrxR activity to be elevated over the activities of those enzymes from chickens fed the selenium-deficient Control diet. The GR activity was not affected by either of the selenium forms or their combination. However, SY supplementation tended to facilitate greater enzyme activity for both GSH-Px and TrxR than did SE or SS. The ATG treatment caused more than a 30% inhibition of GSH-Px activity and more than an 80% inhibition of TrxR activity in the post-mitochondrial supernatant (Table 5), but GR activity was not affected by ATG.

DISCUSSION

Kim and Combs (1993) reported supplemental dietary selenium to a selenium deficient diet induced GSH-Px activity in chickens. Similar data have been reported in male turkeys indicating that GSH-Px activity can be augmented by supranutritional selenium in birds (Sunde and Hadley, 2010; Fischer *et al*., 2008). In this study, both GSH-Px and TrxR activities were increased when supplemental selenium was provided in broiler chicken diets similar to observations made in mammals (Levander *et al*., 1983; Hill *et al*., 1997; Berggren *et al*., 1999; Humann-Ziehank *et al*., 2013). However, selenoenzyme activity in this experiment did not always show a significant selenium-form dependency (Table 3). GR activity was not influenced by either dietary selenium form or concentration in this trial (Table 2 and 5).

Smith *et al*. (2001) compared TrxR activity in mammals and chickens, finding chickens to have extremely low TrxR activities. The low TrxR activities in chickens might indicate low TrxR protein expression or might indicate that chicken TrxR (s) differ significantly from mammalian TrxRs (Liu and Stadtman, 1997). Gowdy (2004) used Western blots and found TrxR protein expression at relatively low levels. All organs expressed a heavy band that was higher in molecular weight (≈70 kDa) than the rat standard (≈56-58 kDa), but also had a light band that was approximately ≈56-58 kDa, along with a light band of a higher molecular weight and a light band ≈35 kDa, which suggested that several TrxR isoforms might exist in chickens. The ≈70 kDa molecular weight band might be attributed to glycosylation of chicken TrxR, but even if this were to be the case, the active sight containing the selenocysteine should have been conserved in the chicken as it is in other species (Gromer *et al*., 2003). These observations also suggest that the chicken expresses TrxR isoforms similar to TrxR1 and TrxR2 found in mammals, but additional research is required. Rozell *et al*. (1985) observed that the thioredoxin-thioredoxin reductase system was widely distributed among tissues and organs and exhibits significant

variation among cell types. In this current investigation, TrxR activity was found in all organs, but TrxR activities among those organs was variable (Table 3) and both selenium forms and their combination elevated ($p \leq 0.05$) TrxR activity over that found in selenium deficient controls. However, neither of the two dietary selenium forms nor their combination altered plasma and red blood cell TrxR activity. It has been suggested that plasma TrxR is a secreted form, possibly TrxR1, which might not reflect tissue TrxR activity (Soderberg *et al*., 2000; Oberley *et al*., 2001). Yet, the red blood cells in this investigation did not show altered TrxR activity in response to dietary selenium supplementation and we do not have an explanation for the lack of TrxR responses to supplemental selenium. It has been reported that TrxR1 activity in human red blood cells is responsive to blood selenium concentrations (Karunasinghe *et al*., 2006), especially organic selenium (Karunasinghe *et al*., 2013).

The subcellular ultrastructural localization of TrxR is associated with all intracellular structures in rat hepatic cells (Rozell *et al*., 1988). Rigobello *et al*. (1998) measured TrxR activity in rat hepatic subcellular structures and noted that there were different isoforms of the enzyme in the mitochondria and the cytosol fractions. Additionally, TrxR activity was reported to be higher in the matrix of the mitochondria than the mitochondrial pellet (Rigobello *et al*., 1998) similar to the results of this current investigation in chickens. Oberley *et al*. (2001) have reported that TrxR activity is found in all subcellular fractions, but that activity is cell-type-specific localized with TrxR2 being predominantly within mitochondria and TrxR1 in the cytosol of rat renal cells. The distribution of TrxR in subcellular structures is strategically placed indicating that the thioredoxin-TrxR system has a role to play in protein processing, secretion and formation of protein disulfides (Rozell *et al*., 1985, 1988). Ejima *et al*. (1999) reported higher TrxR activity in the cytosol (90%) than in the mitochondria (10%) of human placental cells. However, Chen *et al*. (2002) found higher TrxR activity in the mitochondria, lysosome and microsome than in the cytosol in the human liver.

The post-nuclear and post-mitochondrial supernatants in this study with chickens are primarily cytosolic fractions, exhibiting TrxR activities that were comparable to the liver tissue homogenate TrxR activity and with higher ($p \leq 0.05$) activity than in the mitochondrial pellet. The mitochondrial lysate had higher ($p \leq 0.05$) TrxR activity than did the mitochondrial pellet but was comparable to the cytosolic fractions. These results are different from subcellular TrxR activity results in the rat (Rozell *et al*., 1985, 1988) and human tissue (Chen *et al*., 2002; Ejima *et al*., 1999). In this case with chickens, mitochondrial and cytosolic fractions had TrxR activities that were equivalent. There was a higher TrxR activity in the nuclear pellet and presumably this activity was associated with the thioredoxin-thioredoxin reductase system functions in the maintenance of DNA and in the transcription of DNA (Mustacich and Powis, 2000; Powis and Montfort, 2001), which suggested that the thioredoxin-TrxR system in chickens is very important.

The TrxR activity was low in selenium deficient control-fed chickens, but in chickens supplemented with selenium, the TrxR activity was elevated significantly by both selenium forms in many of the tissues. Berggren *et al*. (1999) showed that TrxR activity was increased significantly with dietary selenium supplementation. Selenium-dependent increase in TrxR activity might be due to an increase in the specific activity of the enzyme, which should result in better antioxidant protection (Allan *et al*., 1999). Our observations support this conclusion as it applies to both TrxR and GSH-Px. Increased TrxR activity is associated with the amount of selenium incorporated into TrxR (Gladyshev *et al*., 1996). TrxR isolated from human placental cells had a higher rate of selenocysteine incorporation when more selenium was added to the cell culture media (Gladyshev *et al*., 1996). Mahmoud and Edens (2003) have confirmed that GSH-Px activity was increased to a higher rate with organic selenium dietary supplementation than with sodium selenite dietary supplementation and Edens (2002) has reviewed research reports that show SY to be more readily available for selenoprotein synthesis than sodium selenite.

Enzymes, in which selenium forms the active site of an enzyme, are inhibited by ATG (Chaudiere and Tappel, 1984; Hill *et al*., 1997). Hu *et al*. (1988) reported that repeated administration of ATG to rats over an 8-wk period decreased platelet, kidney and liver GSH-Px activity. Thus, administration of ATG can have some of the same effects as selenium deficiency on selenocysteine-containing enzymes *in vivo*. In chickens, ATG in mM concentrations has been shown to inhibit GSH-Px in the cytoplasm and in mitochondria (Mercurio and Combs, 1985, 1986; Marchionatti *et al*., 2008). Smith *et al*. (1999) noted that as little as 0.025 mg ATG/g body weight (somewhat less than 0.1 μM ATG/g body weight used in this study) inhibited significantly hepatic TrxR in mice. In this study, 0.1 μM/g BW of ATG also caused a significant inhibition of GSH-Px (more than 30%) and TrxR (more than 80%) at 6 hours after an intra-abdominal injection of ATG (Table 5). These results show that the chicken selenoenzymes, GSH-Px and TrxR, are sensitive to ATG inhibition.

The observations made in this investigation show that TrxR activity, over a relatively broad range, is found in all tissues examined in broiler chickens. Activity of TrxR as well as that of GSH-Px are selenium dependent and in this investigation both inorganic and organic selenium dietary supplementation caused an equivalent elevation in the activities of these selenoenzymes. Subcellular

distribution of TrxR activity was found in association with the cytosolic, nuclear pellet and mitochondrial fractions and TrxR activity was found to be equivalent in the mitochondrial fraction and the cytosolic fraction, differing from the condition found in mammals. Preliminary evidence suggests that at least two different isoforms of TrxR are found in the mitochondrial fraction (TrxR2) and in the cytosolic fraction (TrxR1), but additional research is required to elucidate this point in chickens. Aurothioglucose was shown to inhibit TrxR activity by more than 80% and GSH-Px activity by more than 30% in this investigation. Dietary selenium form did not alter the aurothioglucose inhibition of these enzymes. These data indicate that selenium supplementation in broiler chickens has a beneficial effect on multiple selenoproteins that can alter the health status of the bird.

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