Glutathione and Respiratory Chain Complex Activity in Duodenal Mitochondria of Broilers with Low and High Feed Efficiency

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ABSTRACT We previously observed increased reactive oxygen species (ROS) production in intestinal mitochondria obtained from broiler breeder males with low feed efficiency (FE, gain-to-feed). Because antioxidants are critical for combating ROS-mediated oxidative stress and preserving mitochondrial function, the objectives of this study were 1) to determine levels of reduced glutathione (GSH), a major antioxidant in mitochondria, 2) to measure activities of GSH recycling enzymes: GSH peroxidase and GSH reductase, and 3) to establish relationships between antioxidants and respiratory chain complex activities (complexes I, II, III, IV, and V) in broiler breeder males with low and high FE. Duodenal mitochondria were isolated from broilers with low (0.62 ± 0.01, n = 8) and high (0.80 ± 0.01, n = 8) FE. Activities of respiratory chain complexes, GSH peroxidase, and GSH reductase, and levels of GSH were measured by UV spectrophotometry. There were no differences in GSH peroxidase or reductase activities or in individual complex activities between groups but GSH levels tended to be higher (P = 0.075) and oxidized to reduced glutathione ratio tended to be lower (P = 0.077) in broilers with high FE. Regression analysis revealed significant correlations (P ≤ 0.05) between mitochondrial GSH and activities of complexes II, IV, and V with R² values of 0.35, 0.56, and 0.49, respectively. These data suggest that GSH may be important in maintaining or enhancing the activity of certain respiratory chain complexes and could be involved in the phenotypic expression of feed efficiency in broilers.

(Key words: broiler, complex activity, feed efficiency, glutathione, oxidative stress)

INTRODUCTION Feed efficiency (FE, gain:feed) or feed conversion ratio (feed:gain) remains an important trait in commercial animal breeding programs because feed cost represents 50 to 70% of the cost of livestock production. Genetic selection has resulted in 250 to 300% improvement in body weight and FE in 1991 and 2001 broiler strains compared with a 1957 randombred control population (Havenstein et al., 1994, 2003; Chapman et al., 2003). However, as much as 10% variation in growth and FE still exists within broiler lines (Emmerson, 1997). Because mitochondria generate 90% of the cellular energy, we hypothesize that some of the variation in FE may be due to inefficiencies in mitochondrial function. Recent evidence suggested that mitochondrial function or biochemistry, without breed and dietary effects, might be associated with FE in broilers (Bottje et al., 2002; Iqbal et al., 2004; Ojano-Dirain et al., 2004) and in rats (Lufz and Stahly, 2003).

The respiratory chain, located at the inner mitochondrial membrane, consists of 5 multisubunit enzyme complexes: Complex (Cx) I [nicotinamide adenine dinucleotide (NADH):ubiquinone reductase], Cx II (succinate:ubiquinone reductase), Cx III (ubiquinol:cytochrome c reductase), Cx IV (ferrocytochrome c oxidoreductase), and Cx V (ATP synthase or ATPase) (Figure 1). Proton-motive force, generated by the translocation of protons from the matrix to the intermembrane space during electron transport, provides the energy for ATP synthesis (Lehninger et al., 1993). However, the respiratory chain has also been recognized as a major site of reactive oxygen species (ROS) production and, therefore, is a major source of endogenous oxidative stress. Increased ROS may overwhelm the cell’s antioxidant protection and can damage critical structures in the cell including proteins, DNA, and lipids (Yu, 1994). Elevated ROS may also induce cell death (Miwa et al., 2000). Studies

Abbreviation Key: Cx = complex; DTNB = 5,5'-dithiobis (2-nitrobenzoic acid); FE = feed efficiency; GPx = glutathione peroxidase; GR = glutathione reductase; GSH = reduced glutathione; GSSG = oxidized glutathione; NADH = nicotinamide adenine dinucleotide; NADPH = nicotinamide adenine dinucleotide phosphate; ROS = reactive oxygen species.
have indicated that increased ROS due to site-specific defects in the mitochondrial respiratory chain in muscle, liver, and intestine, appeared to contribute to phenotypic expression of low FE in broilers (Bottje et al., 2002, 2004; Ojano-Dirain et al., 2004) and was also implicated in pulmonary hypertension syndrome (Iqbal et al., 2001; Cawthon et al., 1999, 2001; Tang et al., 2002).

Cells possess both enzymatic (e.g., superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic (e.g., glutathione, ascorbic acid, and vitamin E) antioxidants that protect against oxidative injury. Thus, injuries caused by increased ROS are not evident unless the potency of antioxidative defense is exhausted (Augustin et al., 1997). Glutathione is the major endogenous antioxidant in cells and mitochondria (Griffith and Meister, 1985; Märtensson et al., 1993). Glutathione exists in a reduced (GSH) or oxidized (GSSG; glutathione disulfide) form and the ratio of GSSG to GSH is used as an indicator of oxidative stress (Kidd, 1997). The glutathione reduction-oxidation (redox) system consists of GSH and the GSH recycling enzymes, GSH peroxidase (GPx), and GSH reductase (GR) (Meister, 1984). The enzyme GPx metabolizes peroxides (e.g., H$_2$O$_2$) using reducing equivalents from GSH and catalyzes the reaction as shown in equation 1:

\[
2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2 \text{H}_2\text{O} \quad [1]
\]

Low levels of GSSG are maintained by GR, which utilizes reduced nicotinamide adenine dinucleotide phosphate (NADPH$_2$) to reduce GSSG to GSH as shown in equation 2:

\[
\text{GSSG} + \text{NADPH}_2 \rightarrow 2 \text{GSH} + \text{NADP}^+ \quad [2]
\]

The glutathione redox system is a vital defense mechanism of mitochondria against free radical damage as mitochondria lack catalase (Märtensson et al., 1990), γ-glutamyl synthetase (Meister, 1984), and the ability to export GSSG (Olafsdottir and Reed, 1988). Märtensson et al. (1990) noted that the mitochondrial GSH transport system might even be designed to efficiently conserve mitochondrial GSH at the expense of cytosolic GSH.

As increased H$_2$O$_2$ production in low FE mitochondria (Bottje et al., 2002; Ojano-Dirain et al., 2004) may upregulate or change antioxidant enzyme activity (Yu, 1994; Iqbal et al., 2002) and may compromise the functionality of the respiratory chain complexes (Cardoso et al., 1999), the objectives of this study were to: 1) determine the levels of GSH and GSSG, 2) measure activities of the GSH recycling enzymes, GR and GPx, and 3) establish relationships between mitochondrial GSH and respiratory chain complex activities in duodenal mitochondria isolated from broilers with low and high FE.

**MATERIALS AND METHODS**

**Birds and Sampling**

Male broilers with the highest or lowest FE (n = 8/group) were selected from a group of 100 breeder replace-
ment stock\(^3\) tested for FE. Feed efficiency in birds was measured from wk 6 to 7 only and not over the entire 7-wk period. The birds were color-coded and transported to the University of Arkansas, where they were housed in similar cages (51 × 51 × 61 cm) and environmental conditions (25°C; 15L:9D) and fed the same diet provided during the FE trial (20.5% protein, 3,280 kcal/kg). Birds were provided free access to feed and water. After a 5-d acclimation period, one bird at a time was randomly selected for sampling and euthanized with an overdose of sodium pentobarbital by i.v. injection into the caudal tibial vein. Birds with leg problems or those that did not regain appetite after the acclimation period were culled and were not sampled for biochemical assays. The birds used in this study were the same as those used in the study of Iqbal et al. (2004). The duodenal loop was excised and mucosa was carefully removed from the serosa as previously described (Ojano-Dirain et al., 2004). Mucosa was snap-frozen in liquid nitrogen and stored at −80°C for isolation of mitochondria. Data were obtained for weight, length, and diameter of the duodenal loop, and mucosa weight. Experiments were conducted without knowledge of the FE data until completion of the study.

### Duodenal Mitochondria Isolation

Mitochondria were isolated by differential centrifugation as described by Lawrence and Davies (1986) with modifications (Ojano-Dirain et al., 2004). Briefly, approximately 10 g of mucosa was incubated with dimethylaminoethyl-cellulose suspension [8 g of dimethylaminoethyl-cellulose in 80 mL of isolation medium A (70 mM sucrose, 220 mM mannitol, 2 mM HEPES, 0.5 mM EGTA (ethylene glycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid), 0.1 mM phenylmethylsulfonyl fluoride, and 0.37 g of fatty acid-free BSA/100 mL, pH 7.4), 175 units/ml heparin, and 1mM dithiothreitol]. After 2 min, 50 mL of isolation medium A was added and the mucosal cells were homogenized and centrifuged at 750×g for 10 min. The supernatant and mitochondria were centrifuged for 7 min at 9,800 and 12,100×g, respectively. The mitochondrial pellet was enriched (12,100×g for 7 min) in isolation medium B (70 mM sucrose, 220 mM mannitol, 2 mM HEPES, and 1.2 g of fatty acid-free BSA/100 mL, pH7.4). The final mitochondrial pellet was suspended in isolation medium B and stored at −80°C for biochemical assays. All procedures were carried out at 4°C.

Mitochondrial protein was determined using a Bradford assay\(^4\) according to Lawrence and Davies (1986). The purity of isolated mitochondria was evaluated by measuring the activity of citrate synthase, a mitochondrial marker, according to Srere (1969) with modifications.

### Enzyme Activities

**Antioxidants Assay**

Enzyme activities of GR and GPx, and levels of GSH and GSSG were measured using a 96-well plate spectrophotometer (PowerWave X 340).\(^5\) Values were corrected for path length of the 96-well plate and with the appropriate blanks.

**Reduced and Oxidized Glutathione.** The amounts of total glutathione (2 GSH + GSSG, in GSH equivalents) and GSSG were quantified via an enzymatic recycling assay that uses GSH, GR, and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) according to Griffith (1980) and Anderson (1985) with modifications. For total glutathione, 200 µL of mitochondria (~1.6 mg of protein/mL) was deproteinized in 400 µL of 10% 5-sulfosalicylic acid (10,000 ×g for 10 min). An 80-µL aliquot of the resulting supernatant was collected and neutralized with 5 M KOH. Standard solutions were prepared with known amounts of GSH. Glutathione reductase (0.266 units) was added to each well with 140 µL of the reaction buffer (143 mM NaPO₄, 6.3 mM EDTA, pH 7.5, and 0.3 mM NADPH). The plate was incubated at 30°C for 5 min, after which 20 µL of sample or standard solutions (both in triplicate) were added to each appropriate well. The mixture was incubated at 30°C for 10 min, and then 20 µL of 6 mM DTNB was added to each well. The plate was incubated for 3 min at 30°C and the reaction was recorded. The rate of thionitrobenzoic acid formation at 412 nm was proportional to the sum of glutathione (Griffith, 1980). The sample preparation and assay for GSSG was similar to that of total glutathione. Oxidized glutathione was selectively determined by assaying samples in which GSH was not detected, by prior derivatization of GSH with 2-vinyl-pyridine. Total glutathione and GSSG were calculated from the standard calibration curves, and GSH was calculated by subtracting GSSG from total glutathione.

**GR Activity.** The activity of GR (EC 1.6.4.2) was measured as a decrease in absorbance for 5 min and 25°C at 340 nm according to Tabata and Floyd (1994) with modifications (Iqbal et al., 2002). Mitochondria (50 µL) were incubated for 5 min at 21°C in 140 µL of reaction buffer (36 mM KH₂PO₄, 2 mM EDTA at pH 7.4, and 0.3 mM GSSG). The reaction was initiated by adding 0.1 mM NADPH; activity was expressed as units of GR per milligram of protein.

**GPx Activity.** The activity of GPx (EC 1.11.1.9) was determined according to Lawrence and Burk (1976) with modifications (Iqbal et al., 2002). The activity was measured as a decrease in absorbance at 340 nm for 5 min at 25°C based on a coupled reaction with GR in the presence of GSH and H₂O₂. Mitochondria (50 µL) were added to each well containing 140 µL of 25 mM KH₂PO₄, 0.5 mM EDTA, pH 7.4; 0.5 mM NaNO₃, 0.3 mM NADPH, 0.64 units of GR, and 1 mM GSH. The reaction was initiated by adding 0.1 mM H₂O₂. Values were corrected for nonenzymatic oxidation of GSH and NADPH by H₂O₂; GPx activity was expressed as units per milligram of protein using 6.22 mM⁻¹cm⁻¹ extinction coefficient.
Respiratory Chain Complex Activities

The activity of the respiratory chain complexes was assessed by UV spectrophotometry as described by Barrientos (2002), Cardoso et al. (1999), and Ragan et al. (1987) with modifications. All assays were performed in duplicate at 37°C in a 96-well plate in a final volume of 0.215 mL. Activities are expressed in units per milligram of mitochondrial protein.

**Complex I Activity.** The activity of Cx I was assayed as a decrease in absorbance at 340 nm by following the oxidation of reduced nicotinamide adenine dinucleotide (NADH). Mitochondria (25 to 40 µg of protein) were incubated at 37°C for 2 min in 176 µL of Millipore H₂O. Reaction medium [50 mM Tris-HCl with 5 mg/mL BSA, pH 8.0; 240 µM KCN, 4 µM antimycin A, and 48 µM 2,6-dichlorophenolindophenol] was added and the reaction was initiated with the addition of 0.75 mM rotenone. The difference in the decrease in absorption due to NADH oxidation in the absence and presence of rotenone represents the rotenone-sensitive Cx I activity and was quantified using an extinction coefficient of 6.22 mM⁻¹cm⁻¹.

**Complex II Activity.** Complex II activity was determined by following the secondary reduction of 2,6-dichlorophenolindophenol by ubiquinone-2 at 600 nm. Mitochondria (25 to 40 µg of protein) were added to a buffer containing 50 mM KH₂PO₄, 0.10 mM EDTA, and 18 mM succinate, and incubated for 3 min. Rotenone (5 µM), 0.2 mM ATP, 100 µM 2,6-dichlorophenolindophenol, and 80 µM ubiquinone-2 were added and the activity was recorded for 5 min, and 1 mM thenoyl trifluoracetate was added to inhibit Cx II activity. The activity of Cx II was determined using the thenoyl trifluoracetate-sensitive rate and a molar extinction coefficient of 21 mM⁻¹cm⁻¹.

**Complex III Activity.** The assay was performed at 550 nm by monitoring the rate of reduction of cytochrome c by ubiquinol-2. In a medium containing 10 mM KH₂PO₄ (pH 7.8), 2 mM EDTA, 5 mM MgCl₂, 1 mg/mL BSA, 240 µM KCN, 4 µM rotenone, 200 µM ATP, 0.6 mM n-dodecyl-β-d-maltoside, and 40 µM oxidized cytochrome c, mitochondria (25 to 40 µg of protein) were added. The reaction was initiated by adding 80 µM ubiquinol-2, and the increase in absorbance was monitored for 80 s. The nonenzymatic reduction of cytochrome c was measured after addition of 4 µM antimycin A; the specific activity of Cx III was calculated by subtracting the nonenzymatic rate. An extinction coefficient of 19.2 mM⁻¹cm⁻¹ was used.

**Complex IV Activity.** The activity of Cx IV was measured by following the oxidation of reduced cytochrome c (cytochrome c was reduced with sodium dithionite) as a decrease in absorbance at 550 nm. The reaction medium contained 10 mM KH₂PO₄, 250 mM sucrose, 1 mg/mL BSA, 240 µM KCN, and mitochondria (25 to 40 µg of protein). The reaction was initiated by adding 10 µM reduced cytochrome c, and the reaction was followed for 60 s. The specific activity was calculated by using 21 mM⁻¹cm⁻¹ extinction coefficient.

**Complex V Activity.** The activity of Cx V was determined by following the reduction of NADH with lactate dehydrogenase and pyruvate kinase as coupling enzymes. Millipore H₂O (165 µL) and 45 µL of reaction medium (50 mM Tris, pH 8.0, 20 mM MgCl₂, 50 mM KCl, 15 µM carboxyl m-chlorophenylhydrazone cyanide, 5 µM antimycin A, 10 mM phosphoenolpyruvate, 2.5 mM ATP, 20 units of lactate dehydrogenase, 20 units of pyruvate kinase, 1 mM NADH, and 5 mg/mL BSA) were incubated at 37°C for 5 min. Mitochondria (25 to 40 µg of protein) were added and incubated for 30 s, and the reaction was followed for 3 min with and without oligomycin (3 µM). The oligomycin-sensitive ATPase activity was calculated with an extinction coefficient of 6.22 mM⁻¹cm⁻¹.

### Statistical Analyses

Data were analyzed by regression analysis and 1-way ANOVA using JMP 5.0 statistical software. Means were separated by Student’s t-test and data are presented as the mean ± SEM. A probability level of P ≤ 0.05 was considered significant unless stated otherwise.

### RESULTS AND DISCUSSION

The digestive and absorptive capacity of the small intestine influences the supply of nutrients to all other tissues in the body (Cant et al., 1996). The efficiency of nutrient utilization is vital not only for postabsorptive and demand tissues (e.g., muscle) but also to the optimal functioning

<table>
<thead>
<tr>
<th>Variables</th>
<th>High FE (n = 8)</th>
<th>Low FE (n = 8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-wk BW, g</td>
<td>2,433 ± 32</td>
<td>2,450 ± 26</td>
<td>0.69</td>
</tr>
<tr>
<td>7-wk BW, g</td>
<td>3,349 ± 49</td>
<td>3,134 ± 52</td>
<td>0.009</td>
</tr>
<tr>
<td>Feed intake, g</td>
<td>1,169 ± 76</td>
<td>1,106 ± 80</td>
<td>0.58</td>
</tr>
<tr>
<td>FE (gain to feed), g/g</td>
<td>0.80 ± 0.01</td>
<td>0.62 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FCR* (feed to gain), g/g</td>
<td>1.27 ± 0.05</td>
<td>1.62 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM.

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*Bio-Tek Instruments, Inc., Winooski, VT.
**Millipore Corp., Bedford, MA.
*SAS Institute Inc., Cary, NC.
of the gastrointestinal tract, because 15 to 25% of the whole bird energy requirement is spent by the gastrointestinal tract for cell renewal and nutrient absorption (Croom et al., 1999). It is suggested that if more than 25% of the bird’s energy requirements were utilized to support gastrointestinal tract function, the utilization of energy for growth would be greatly decreased (Cant et al., 1996; Croom et al., 1999). Croom et al. (1999) suggested that intensive genetic selection for growth performance parameters in poultry might have uncoupled intestinal nutrient absorption from increased postabsorptive nutrient demands. Although a number of studies have evaluated possible interventions to improve nutrient utilization or decrease energy expenditures of the gastrointestinal tract (e.g., Bird et al., 1996; Cant et al., 1996; Croom et al., 1999), our interest is in the association of intestinal mitochondrial function and the phenotypic expression of feed efficiency. Jha et al. (2000) reported specific defects at complexes I and III (Ojano-Dirain et al., 2004) and tendency of higher mitochondrial ROS values of PC12 cells with a concomitant decrease in mitochondrial function. Therefore, it may still be possible that the higher ROS production and reduced coupling (Ojano-Dirain et al., 2004) and tendency of higher oxidative stress (in the current study) in low FE duodenal broilers, reared in the same environment, and fed the same diet. Because mitochondria are responsible for generating cellular energy (ATP) that fuels nutrient digestion, absorption, and cell renewal, inefficiencies in intestinal mitochondrial function may reduce the efficiency of converting feed into demand tissues or eviscerated body mass.

Ojano-Dirain et al. (2004) reported that duodenal mitochondria isolated from low-FE broilers have higher basal respiratory chain complex activity (in units/mg of mitochondrial protein) of duodenal mitochondria from broilers with low and high feed efficiency (FE)1 2004), no differences were observed between groups in physical attributes of the duodenum such as weight, length, diameter, area, and mucosa weight (Table 2). There were no differences in mitochondrial protein and citrate synthase activity between high and low FE mitochondria (Table 3).

The amount of GSH, a major antioxidant in mitochondria, tended to be lower (P = 0.075) in low FE mitochondria (Table 3). There were no differences in the amount of GSSG, but the GSSG/GSH ratio (an index of oxidative stress), tended to be higher (P = 0.077) in the low FE mitochondria, which indicated that low FE birds might have a relatively higher degree of oxidative stress compared with the high FE group. This finding supports earlier observations in which low FE mitochondria exhibited higher ROS production (Ojano-Dirain et al., 2004). The activities of the GSH recycling enzymes, GPx and GR, were not different between high and low FE mitochondria (Table 3). These results contrast slightly with those of Iqbal et al. (2002) in which liver mitochondria from broilers with pulmonary hypertension and higher ROS production showed higher GPx activity but no difference in GR activity compared with control birds. Care should be taken, however, in making a direct comparison between the results of this study and those of Iqbal et al. (2002) because the degree of oxidative stress in pathological conditions of pulmonary hypertension may differ significantly from that in the healthy birds in the present study that differed only in their phenotypic expression of feed efficiency. Jha et al. (2000) reported that lowering GSH levels caused increased cellular and mitochondrial ROS values of PC12 cells with a concomitant decrease in mitochondrial function. Therefore, it may still be possible that the higher ROS production and reduced coupling (Ojano-Dirain et al., 2004) and tendency of higher oxidative stress (in the current study) in low FE duodenal broilers with low and high FE.

Growth performance data (Table 1) shows that FE (g of gain/g of feed) was 0.80 ± 0.01 and 0.62 ± 0.01 for high- and low-FE birds, respectively. The difference in FE or feed conversion ratio (g of feed/g of gain) was mainly a function of higher BW gain (P = 0.01) in the high FE group as there were no differences in feed intake (P = 0.73), which concurs with previous studies (Bottje et al., 2002; Ojano-Dirain et al., 2004). Similar to a previous study (Ojano-Dirain et al.,

<table>
<thead>
<tr>
<th>Variables</th>
<th>High FE (n = 7)</th>
<th>Low FE (n = 5)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Mitochondrial protein, mg/mL</td>
<td>1.58 ± 0.13</td>
<td>1.61 ± 0.20</td>
<td>0.90</td>
</tr>
<tr>
<td>Citrase synthase activity, units/mg of protein</td>
<td>362 ± 32</td>
<td>332 ± 32</td>
<td>0.90</td>
</tr>
<tr>
<td>GSH, µmol/mg</td>
<td>1.5 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.075</td>
</tr>
<tr>
<td>GSSG, µmol/mg</td>
<td>0.10 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.44</td>
</tr>
<tr>
<td>GSSG/GSH ratio</td>
<td>0.070 ± 0.006</td>
<td>0.093 ± 0.01</td>
<td>0.077</td>
</tr>
<tr>
<td>GPx activity, units/mg of protein</td>
<td>32.5 ± 2.2</td>
<td>31.2 ± 7.0</td>
<td>0.85</td>
</tr>
<tr>
<td>GR activity, units/mg of protein</td>
<td>0.89 ± 0.08</td>
<td>1.05 ± 0.13</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± SEM.
mitochondria could be influenced by the amount of GSH or other antioxidants.

The activities of the individual respiratory chain complexes (I, II, III, IV, and V) were not different between the high- and low-FE duodenal mitochondria (Table 4). The absence of differences in the activity of the respiratory chain complexes observed in this study is in contrast to earlier observations (Bottje et al., 2002). In addition, because the birds used in this study were the same birds used by Iqbal et al. (2004), who reported differences in complex activities of breast muscle mitochondria, the contrasting findings in duodenal mitochondria may suggest the unique dynamics between different tissues.

Regression analysis revealed positive correlations (P ≤ 0.05) between mitochondrial GSH and activities of Cx II, IV, and V, with R^2 values of 0.35, 0.56, and 0.49, respectively. (Figure 2). The regression lines for GSH levels and activities of Cx I to V were not different between high and low FE mitochondria (P = 0.14, 0.38, 0.24, 0.28, and 0.32, respectively). These observations are in agreement with studies indicating that GSH levels are critical in maintaining or protecting respiratory chain complex activity from oxidation. For example, Cardoso et al. (1999) reported that the addition of GSH enhanced the activity of Cx II, III, and V of synaptosome mitochondria subjected to oxidative stress. Bolaños et al. (1996) also demonstrated that depletion of neuronal GSH by incubation with either S-nitroso-N-acetylpenicillamine or L-buthionine S,R-sulfoximine resulted in a marked decrease in Cx I, II-III, and IV activities. Jha et al. (2000) also reported that depletion of GSH in PC12 cells decreased Cx I activity but that there was no decrease in the activity of Cx II-III and V. In contrast, Cx I activity of glial cells treated with L-buthionine S,R-sulfoximine for 72 h was 2-fold higher compared with control cells but complex II-III and IV activities were unaltered (Vasquez et al., 2001). Finally, Augustin et al. (1997) reported that mitochondria are not damaged by ROS as long as the mitochondria are in an energized state in which ROS production is minimized. These studies demonstrate that susceptibility to oxidative insults may vary between tissues or cells; they also illustrate the complexity of evaluating the physiological onset of oxidative damage or depletion of mitochondrial antioxidant capacity in vitro. However, the positive correlation between levels of total GSH and activities of Cx II, IV, and V in this study and the results of other studies (e.g., Bolaños et al., 1996; Cardoso et al., 1999; Jha et al., 2000) suggest that GSH has an important role in protecting the respiratory chain complexes against oxidative damage. An inverse correlation between GSSG/GSH ratio and Cx I to V activities was observed, but regression lines were not significant (data not shown). In conclusion, it appears that differences in ROS production and GSH levels may be responsible, in part, for the observed differences in feed efficiency between the high- and low-FE broilers. These observations add to our efforts in understanding the cellular and biochemical mechanisms associated with feed efficiency.

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