INVITED REVIEW

Feed Efficiency and Mitochondrial Function

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ABSTRACT  Studies have been conducted in our laboratory to assess differences in mitochondrial function and biochemistry in male broilers with high and low feed efficiency (FE) from the same genetic line and fed the same diet. Mitochondria obtained from broilers with low FE exhibited greater uncoupling of the electron transport chain (ETC) that was apparently due to site-specific defects in electron transport resulting in higher amounts of reactive oxygen species (ROS) compared with high FE mitochondria. Higher amounts of ROS production in Low FE mitochondria were likely responsible for higher protein carbonyl levels, indicative of higher protein oxidation compared with High FE mitochondria and tissue. In turn, higher protein damage in Low FE mitochondria may have contributed to lower activity of electron transport chain complexes relative to values observed in high FE mitochondria. Low FE mitochondria did not exhibit a compromised ability to carryout oxidative phosphorylation, and although there were differences in expression of certain electron transport chain proteins, there was nothing that would indicate that differences in coupling and respiratory chain activity could be due to a general decrease in protein expression between low and high FE mitochondria. The results of these studies provide insight into understanding cellular mechanisms associated with the phenotypic expression of feed efficiency in broilers.

Key words: broiler, feed efficiency, mitochondrial function

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INTRODUCTION

An overview of the history of mitochondria was recently provided by Nisoli et al. (2004). It is interesting that the first description of mitochondria as discrete organelles occurred over 150 years ago by a cytologist, Kölliker, in 1850 (see Lehninger, 1965) and that the importance of mitochondria in the production of energy by oxidative phosphorylation for the cell was first reported more than 50 years ago (Kennedy and Lehninger, 1949). Nisoli et al. (2004) indicated that the construction of the intricate components of the complete mitochondria as well as regulation of biogenesis and growth of existing mitochondria is complex and controlled by the activation of specific transcription factors and cell signaling pathways. Thus, understanding of mitochondrial physiology has obviously advanced in 50 yr, but there is still much to be explored. This paper will provide a short overview of mitochondrial function and physiology, and will then focus on studies being conducted that provide a linkage between mitochondrial function and biochemistry with the phenotypic expression of feed efficiency in broilers.

MITOCHONDRIAL FUNCTION AND BIOCHEMISTRY

The oxidative phosphorylation system consists of 4 multiprotein complexes (I to IV) and ATP synthase (complex V) (Lehninger et al., 1993). Electrons enter the electron transport chain (ETC) from nicotinamide adenine dinucleotide, reduced (NADH) or flavine adenine dinucleotide, reduced (FADH₂) linked substrates at complex I and complex II, respectively. Electron movement in the ETC to the terminal electron acceptor, O₂, is coupled to proton pumping into the intermembrane space. The resulting proton motive force drives ATP synthesis [from ADP and Pᵢ] as protons move back to the matrix through ATP synthase. Mitochondrial inefficiency may occur because of electron leak from the respiratory chain. Rather than being completely reduced to water, 2 to 4% of oxygen consumed by mitochondria may be incompletely reduced to reactive oxygen species (ROS) such as superoxide and H₂O₂ due to univalent reduction of oxygen by electrons that leak from the respiratory chain before they reach the terminal electron acceptor (Boveris and Chance, 1973; Chance et al., 1979). The mitochondrial formation of ROS makes the mitochondrion a major source of oxidative stress in the cell. If not metabolized by antioxidants, ROS can cause oxidation of critical biomolecules (e.g., lipids, proteins, and DNA) in the mitochondrion or other parts of the cell, which can lead to further inefficiencies and accentuate additional ROS production.
Increased mitochondrial ROS production has been linked to various metabolic diseases (Fiegel and Shapiro, 1979; Hagen et al., 1997; Kristal et al., 1997; Herrero and Barja, 1998; Lass et al., 1998; Cawthon et al., 2001; Iqbal et al., 2001a; Tang et al., 2002). The use of respiratory chain inhibitors can be employed to identify site-specific defects in electron transport within mitochondria. Whereas electron leak occurs mainly within complexes I or III of the respiratory chain (Turrens and Boveris, 1980; Nohl et al., 1996; Herrero and Barja, 1998), sites of H2O2 production are tissue-dependent (Kwong and Sohal, 1998). For example, in broilers with pulmonary hypertension syndrome, increased ROS production was associated with complexes I and III in heart, muscle, and lung (Iqbal et al., 2001a; Tang et al., 2002) and complex II in liver mitochondria (Cawthon et al., 2001).

Mitochondrial function and biochemistry is dependent upon the careful orchestration of protein synthesis occurring by nuclear (n) DNA encoding as well as synthesis of proteins by a discrete mitochondrial (mt) DNA that encodes 22 transfer RNA, 2 ribosomal RNA, and 13 ETC proteins (Anderson et al., 1981; Desjardin and Morais, 1990). Thus, expression of respiratory chain proteins is under control of both nuclear and mitochondrial DNA (Sue and Schon, 2000). Mitochondrial function also requires the import of hundreds of proteins including ETC proteins synthesized by nuclear DNA (Rabilloud et al., 2002). Some proteins are part of the mitochondrial import machinery, whereas others are needed for expression of its genome and metabolism, and others are important for apoptosis (Liu and Kitsis, 1996), redox cell signaling, and homeostasis (Bogoyevitch et al., 2000; Levonen et al., 2001; Droge, 2002). Consequently, as stated by Rabilloud et al. (2002), mitochondrial function in general, and mitochondrial protein synthesis in particular, depend on the coordinated expression of both mitochondrial and nuclear genomes.

MITOCHONDRIA AND FEED EFFICIENCY

Overview

Feed efficiency (FE) remains an important trait for commercial breeding companies because feed represents 50 to 70% of the cost of raising a bird to market weight. Genetic selection for FE has been responsible for more than 80% of the improvement in feed efficiency in modern broilers (Havenstein et al., 1994, 2003). Because mitochondria are responsible for producing 90% of the energy needed for cells, we have conducted a series of studies to understand relationships of mitochondrial function and biochemistry with the phenotypic expression of feed efficiency in broilers (Bottje et al., 2002, 2004; Iqbal et al., 2004, 2005; Ojano-Dirain et al., 2004, 2005a,b; Tinsley et al., 2004; Lassiter, 2005).

It is well known that genetics and diet have profound influences over mitochondrial function. For example, differences in oxygen utilization rates between breeds of chicken (Mukherjee et al., 1970; Dzwiewiecki and Kolataj, 1976) have been observed and mitochondria have been hypothesized to be part of the basis for heterosis observed in plants (McDaniel and Sarkissian, 1966; Srivistava, 1981), sheep (Wolanis et al., 1980), swine (Dzabo and Wassmuth, 1983), and chicken (Brown et al., 1986). Dietary manipulations of fat and protein levels have been shown to have effects on mitochondrial function (Renner et al., 1979; De Schrijver and Privett, 1984; Toyomizu et al., 1992a,b,c). However, in each of these studies, mitochondrial function was investigated in response to a dietary difference or with respect to different breeds. The studies outlined below will summarize results obtained in our laboratory in which mitochondrial function and biochemistry were determined in a single line of broilers fed the same diet, thus eliminating dietary effects or differences in breed (e.g., slow vs. fast growing or fat vs. lean lines). These studies have helped provide a better understanding of the cellular basis of feed efficiency.

In each of the studies in our laboratory, birds with the lowest or highest FE (6 to 8 per group) were identified within a group of 100 breeder male replacement stock (Bottje et al., 2002). In all studies summarized in this review, the high FE birds exhibited weights similar to low FE birds at the start of the week of the feed efficiency determination but gained more during the week on the same amount of feed as the low FE birds. Typical differences in FE between broilers with low and high FE in these studies is provided in Figure 1 (taken from Bottje et al., 2002). The dependency of BW gain on feed intake is clearly indicated. Feed efficiency (g of gain/g of feed) in this study was 0.64 ± 0.01 and 0.83 ± 0.01 for low and high FE groups, respectively. Tissues that have been investigated include breast muscle (pectoralis superficialis), leg muscle (quadriceps femoris), liver, upper duodenum, heart, and lymphocytes. Mitochondria were isolated by differential centrifugation.
Coupling and Oxidative Phosphorylation in Low FE Mitochondria

Relationships in muscle mitochondrial function and FE in broilers were presented (Bottje et al., 2002). There were no differences in mitochondrial function provided succinate. However, when provided NADH-linked substrates, the respiratory control ratio [RCR, an index of respiratory chain coupling (Estabrook, 1967)] was higher in high FE breast and leg muscle mitochondria compared with low FE mitochondria. These results indicate more efficient coupling of electron transport in high FE than in low FE muscle mitochondria and provide indirect evidence that functional differences (i.e., differences in respiratory chain coupling) in muscle mitochondria between the 2 groups might be due to differences in electron transport associated with complex I. Regression analysis revealed that breast mitochondria RCR values were highly correlated with FE, similar to that reported in rats (Lutz and Stahly, 2003). There were no differences in the ADP/O with either energy substrate. Thus, low FE mitochondria did not exhibit a compromised ability to carry out oxidative phosphorylation.

Studies were also conducted to determine relationships between intestinal mitochondrial function and FE in broilers (Ojano-Dirain et al., 2004). In that study, duodenal mitochondrial function was assessed following repeated additions of ADP; a paradigm of repeated energy demand that revealed mitochondrial dysfunction in pulmonary hypertension syndrome in broilers (Cawthon et al., 1999; Iqbal et al., 2001b). There were no differences in the initial RCR in duodenal mitochondria obtained from broilers with low and high FE provided NADH or FADH2-linked energy substrates, but after a second addition of ADP, tighter coupling (i.e., higher RCR values) was observed in high FE duodenal mitochondria when succinate was provided but not when NADH-linked energy substrates were provided. These findings suggest that there was a defect in electron coupling associated with complex II in low FE duodenal mitochondria. Because low FE duodenal mitochondria provided with NADH-linked energy substrates exhibited a significantly higher ADP/O ratio with the second addition of ADP, the ability to synthesize ATP may actually be superior in low FE duodenal mitochondria to that observed in high FE mitochondria under some conditions. Possibly, there is a greater demand for ATP in low FE mitochondria; for example, increased ATP needed to repair oxidatively damaged proteins (as discussed below).

Increased Mitochondrial ROS Production and Site-Specific Defects in Electron Transport in Low FE Mitochondria

To determine if ROS production plays a role in inefficiencies associated with low FE mitochondria, H2O2 was monitored in freshly isolated mitochondria according to Iqbal et al. (2001a) to assess electron leak and to identify site-specific defects in electron transport in muscle, liver, and intestinal mitochondria (Bottje et al., 2002; Iqbal et al., 2004, 2005; Ojano-Dirain et al., 2004). In these studies, H2O2 production in untreated (no inhibitor) mitochondria represents the basal electron leak. A summary of relative differences in basal ROS production in high and low FE mitochondria provided either NADH- or FADH2-linked energy substrates is shown in Figure 2. Except in leg muscle, these findings indicate a generalized increase in ROS production, suggesting inherently greater oxidative stress in low FE mitochondria.

According to Barja (1999), increased radical production following ETC inhibition indicates a defect in electron transport between the site of inhibition and entry of substrate into the ETC. Thus, an increase in radical production in mitochondria with a specific inhibitor would indicate a site-specific defect at that site of the respiratory chain.

Bottje et al. (2002) reported increased electron leak (H2O2 production) in low FE breast muscle mitochondria following electron transport inhibition of complexes I and III with rotenone and antimycin A, respectively, indicating that these are likely areas of site-specific defects in electron transport contributing to the higher basal H2O2 production in low FE breast muscle mitochondria. No increase in H2O2 production in high FE mitochondria was observed following electron transport inhibition at complexes I and III, suggesting that high FE mitochondria have lower electron leak in vivo. No differences were observed when electron transport was inhibited at complex II or at the Q cycle of complex III. Complex I may also be a potential site of electron leak in low FE leg muscle mitochondria (Bottje et al., 2002).

Electron transport defects were also investigated in duodenal mitochondria (Ojano-Dirain et al., 2004). Similar to that observed in muscle, basal radical production was higher in low FE duodenal mitochondria provided NADH- or FADH2-linked substrates. However, unlike muscle mitochondria, low FE duodenal mitochondria provided with succinate or pyruvate-malate as energy sources exhibited site-specific defects in electron transport at complexes I, II, and III.

Lower Complex Activities in Low FE Mitochondria

With the exception of one study (Ojano-Dirain et al., 2005a), we have observed a general reduction in the activities of complexes I to V of the respiratory chain in low FE mitochondria (Bottje et al., 2002; 2004; Iqbal et al., 2004; 2005; Ojano-Dirain et al., 2005b). Bottje et al. (2002) reported that activities of complexes I and II in breast and leg muscle mitochondria from broilers with low FE were 63 to 79% of the levels of activity observed in high FE broilers. Additional studies by Iqbal et al. (2004, 2005) indicated that complex activities in low FE muscle and liver mitochondria were all significantly lower than in high FE mitochondria. The biggest difference was in complex IV activity with values in the low FE mitochondria that were 44 ± 8 and 59 ± 4% compared with high FE
values in muscle and liver, respectively. These data suggest that a generalized decrease in respiratory chain complex activity in muscle and liver mitochondria is associated with low FE. In contrast to an earlier study (Ojano-Dirain et al., 2005a), Ojano-Dirain et al. (2005b) reported that all respiratory chain complex activities, with the exception of complex IV, were lower in low FE duodenal mitochondria. The reason for differences between these studies is not apparent at this time.

**Oxidative Stress and Complex Activities**

With the evidence of increased ROS production occurring in low FE mitochondria described above, one mechanism that might contribute to the generally lower activity of respiratory chain complexes in low FE mitochondria could be oxidative stress and subsequent damage to critical proteins in the respiratory chain.

Mitochondrial antioxidant protection from ROS includes reduced glutathione (GSH), vitamin E, Mn superoxide dismutase, GSH peroxidase, and GSH reductase (Yu, 1994). Glutathione is vital to mitochondria by metabolizing or indirect interaction with ROS through the action of GSH peroxidase or by donating reducing equivalents directly. Manganese superoxide dismutase converts superoxide to H$_2$O$_2$. In turn, GSH peroxidase uses reducing equivalents of GSH to convert hydroperoxides and lipid peroxides to water or lipid alcohols. Metabolism of H$_2$O$_2$ is particularly important due to the propensity to be converted to the highly reactive hydroxyl radical in the presence of transition metals (e.g., Fe$^{2+}$, Cu$^{2+}$) via the Haber-Weiss and Fenton reactions, respectively. Glutathione reductase is essential in reducing oxidized GSH (GSSG), formed by GSH peroxidase, back to GSH to prevent thiol toxicity in mitochondria (Olafsdottir and Reed, 1988).

Although there were no differences in GSH peroxidase or reductase activities, Ojano-Dirain et al. (2005a) observed that GSH levels were lower ($P < 0.08$) and the GSSG/GSH ratio (an index of oxidative stress) was higher ($P < 0.08$) in low FE compared with high FE duodenal mitochondria. Regression analysis revealed positive correlations between GSH levels and the activities of complexes II, IV, and V. This would suggest that mitochondrial GSH in broilers might protect critical thiol groups in the respiratory chain complexes from oxidative damage as previously reported (Cardoso et al., 1999; Jha et al., 2000).

A major indicator of oxidative damage of proteins is the formation of protein carbonyls. In addition to the consistent finding of increased ROS production in low FE mitochondria, another consistent observation is that of increased protein carbonyl formation in low FE mitochondria or tissue (Figure 3). Preliminary results indicate that there may be increased protein carbonyl levels associated with complex III (Higgins et al., 2004).

**Respiratory Chain Complex Activities and Protein Expression**

Because the activities of respiratory chain complexes may depend on the amounts of protein of the protein subunits within each complex, breast muscle mitochondria from low and high FE broilers were probed with antibodies for specific ETC proteins and their expression, as determined by Western blot analysis. Lower complex
activities in low FE muscle and liver were not due to a general decrease in ETC protein expression because several ETC proteins were expressed at levels equal to or higher than in high FE mitochondria (Iqbal et al., 2004, 2005). Similar findings have been observed in lymphocytes (Lassiter, 2005) and heart muscle (Tinsley et al., 2004). In the duodenum, 6 of 8 nuclear-encoded proteins were higher in low FE mitochondria, whereas 3 of 6 mitochondrial-encoded proteins were higher in high FE mitochondria (Ojano-Dirain et al., 2005b). The expression of the ATP synthase α-subunit was higher in high FE liver and lymphocytes (Iqbal et al., 2005; Lassiter, 2005), lower in high FE compared with low FE duodenal mitochondria (Ojano-Dirain et al., 2005b), and was not different between low and high FE groups in muscle (Iqbal et al., 2004).

In summary, although differences in expression of proteins were observed between low and high FE mitochondria within a tissue, the differences did not hold for any particular ETC protein from tissue to tissue. Thus, differences in expression of mitochondrial proteins between low and high FE mitochondria may depend on whether they are encoded by mitochondrial or nuclear DNA and apparently vary from tissue to tissue. From these findings, it does not appear that there is any decrease in expression of individual proteins in the respiratory complexes that would account for the generalized reduction in respiratory chain complex activities observed in low FE mitochondria.

Another protein that should be mentioned is adenine nucleotide transporter (ANT1). This protein is located on the mitochondrial inner membrane, and is responsible for exchange of ADP and ATP between the mitochondrial matrix and the cytosol (Li et al., 1989). As such, ANT1 is important for energy production, because membranes are impermeable to adenine nucleotides. Indeed, decreased mitochondrial function was observed in AN1 knockout mice that lacked the ability to exchange ADP and ATP between the mitochondria and cytosol (Graham et al., 1997). Thus, it is hypothesized that differences in expression of AN1 could play a role in the phenotypic expression of feed efficiency. The expression of AN1 was higher in low FE muscle (Iqbal et al., 2004) and heart (Tinsley et al., 2004) mitochondria, but not in liver (Iqbal et al., 2005) or duodenum (Ojano-Dirain et al., 2004).

**SYNOPSIS**

Investigation of respiratory chain protein expression in muscle and liver did not reveal a consistent pattern in protein expression between broilers with low and high feed efficiency. Because there are many other proteins that are important for function of mitochondria and the respiratory chain (e.g., transport and scaffolding proteins), it is possible that the differences we seek may lie in this direction; that is, in proteins other than those associated with the respiratory chain. For this reason, we have initiated a proteomics approach directed towards identifying candidate proteins that are differentially expressed according to FE type. We have begun to investigate transcription factors that influence mitochondrial protein synthesis that may be related to feed efficiency such as that reported in rats by Nisoli et al. (2004). Compared with high FE mitochondria, mitochondria obtained from low FE broilers appear to exhibit decreased electron transport chain coupling, increased electron leak with subsequent increased ROS production, increased protein oxidation, and lower respiratory chain complex activities.
We believe that the lower complex activities may be the result of increased protein oxidation. The positive correlation observed between mitochondrial GSH and complex II, IV, and V activity in the duodenum appears to support this hypothesis. Thus, the consistent findings of higher mitochondrial ROS production and protein oxidation suggest that these are important mechanisms contributing to the phenotypic expression of low FE in broilers in our studies. Although we have identified higher ROS production and protein oxidation in broilers with low feed efficiency, the important question of what causes these processes to occur remains unanswered.

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