Association of Mitochondrial Function with Feed Efficiency within a Single Genetic Line of Male Broilers[1]

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ABSTRACT Studies were conducted to determine relationships between feed efficiency and mitochondrial function and biochemistry. After feed efficiency (FE; gain:feed) was determined in broiler breeder males between 6 and 7 wk of age, mitochondria were isolated from breast and leg muscle from birds with high FE (0.83 ± 0.01, n = 6) and low FE (0.64 ± 0.01, n = 7). Respiratory chain coupling, assessed by the respiratory control ratio (RCR), was greater in high FE breast, and leg mitochondria provided NADH-linked, but not FADH-linked, energy substrates. There were no differences, however, in the adenosine diphosphate to oxygen (ADP:O) ratio (an index of oxidative phosphorylation) when mitochondria were provided either energy substrate. Electron leak, as determined by generation of H_2O_2, was greater in the low FE than in high FE breast mitochondria. Electron leak increased following inhibition of electron transport at Complex I (with rotenone) and Complex III (with antimycin A) in low FE but not in high FE breast mitochondria. There were no differences in basal electron leak in leg mitochondria between groups, but H_2O_2 generation was elevated ($P < 0.07$) compared to basal values in low FE leg mitochondria after Complex I inhibition. The activities of Complexes I and II were greater in high FE breast and leg muscle mitochondria compared to those in low FE mitochondria. The results indicate that lower respiratory chain coupling in low FE muscle mitochondria may be due to lower activities of Complexes I and II and defects in electron leak and provide insight into cellular mechanisms associated with the phenotypic expression of feed efficiency in broilers.

(*Key words*: broiler, feed efficiency, mitochondria, electron leak, respiratory chain complex)

2002 Poultry Science 81:546–555

INTRODUCTION

Feed efficiency remains one of the most important traits in commercial animal breeding programs, as feed represents 60 to 70% of the cost of raising an animal to market weight. A 250 to 300% improvement in body weight and feed efficiency was observed in a 1991 broiler strain when compared to a 1957 random bred control population (Havenstein et al., 1994). Despite marked improvement in these traits, there remain significant within- and between-strain variations in growth and feed efficiency that include a 10% variation in broiler crosses for feed efficiency (Emmerson, 1997). As mitochondria are responsible for producing 90% of the energy for the cell, some of the variations in broiler growth performance and phenotypic expression of feed efficiency (Emmerson, 1997) may be due to differences or inefficiencies in mitochondrial function. Indeed, differences in mitochondrial oxygen use have been observed among various breeds of chickens (Mukherjee et al., 1970; Dziewiecki and Kolataj, 1976; Brown et al., 1986), sheep and swine (Wolanis et al., 1980; Dzapo and Wassmuth, 1983), and with various dietary manipulations (Renner et al., 1979; De Schrijver and Privett, 1984; Toyomizu et al., 1992a,b,c). However, there are no reports regarding associations of mitochondrial function and feed efficiency within a single breed of animals fed the same diet.

The respiratory chain/oxidative phosphorylation system of the inner mitochondrial membrane consists of four multiprotein complexes (I to IV) and adenosine triphos-

Received for publication July 17, 2001.

Accepted for publication November 27, 2001.

[1]This research is published with support by the Director of the Agriculture Research Experiment Station, University of Arkansas, Fayetteville, AR, and funded in part by a grant from Cobb-Vantress, Inc., and a grant from USDA-NRI (2001-03443).

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Abbreviation Key: ACR = acceptor control ratio; ADP:O = adenosine diphosphate to oxygen ratio; CII:CI = Complex II to Complex I; DCFH-DA = 2′, 7′-dichlorofluorescin diacetate; DCIP = 2,6 dichloroindophenol; FE = feed efficiency; HEPES = N-[2-hydroxyethylpiperizine]-N′-[2-ethanesulfonic acid]; $O_2\bullet^-$ = superoxide; RCR = respiratory control ratio; ROS = reactive oxygen species; TTFA = thenoyltrifluroacetone

phate (ATP) synthase (Complex V). Electron movement down the respiratory chain to the terminal electron acceptor, oxygen (O_2), is coupled to proton (H+) pumping from the matrix to the intermembrane space. The resulting protonmotive force drives ATP synthesis [from adenosine diphosphate (ADP) and P_i] as protons move back through ATP synthase (Lehninger et al., 1993). Electrons enter the respiratory chain from NADH- or FADH-linked substrates such as glutamate and succinate at Complexes I and II, respectively.

Mitochondrial function can be assessed by polarographic measurement of oxygen consumption (Estabrook, 1967). In the presence of energy substrates, isolated mitochondria exhibit an initial slow rate of oxygen consumption designated State 2 respiration. Addition of ADP stimulates electron transport chain activity and initiates rapid oxygen consumption (State 3 respiration) that is followed by a slower rate of oxygen consumption (State 4 respiration) when ADP levels decline following oxidative phosphorylation to ATP. Functional indices calculated from these rates of oxygen consumption include the respiratory control ratio (RCR) and ADP:O ratio (Estabrook, 1967). The RCR represents the degree of coupling or efficiency of electron transport chain activity and is calculated as State 3 divided by State 4 respiration rate. The ADP:O ratio is the amount of ADP phosphorylated per nanoatom of monomeric oxygen consumed during State 3 respiration and is an index of oxidative phosphorylation.

Mitochondrial inefficiency may occur as a result 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 ($O_2\bullet^-$) and H_2O_2 due to univalent reduction of oxygen by electrons (Boveris and Chance, 1973; Chance et al., 1979). The mitochondrial formation of ROS makes this organelle a major source of oxidative stress in the cell. If not metabolized by antioxidants, oxidation of critical biomolecules (e.g., lipids, proteins, and DNA) in the mitochondrion or cell can lead to further inefficiencies that accentuate additional ROS production.

Increased mitochondrial ROS production has been linked to various metabolic diseases (Fiegal 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., 2001; Tang et al., 2001). 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 Complex I or III of the respiratory chain (Turrens and Boveris, 1980; Nohl et al., 1996; Herrero and Barja 1998), Kwong and Sohal (1998) demonstrated that sites of H_2O_2 production are tissue dependent. The findings of Kwong and Sohal (1998) may explain in part the findings of increased ROS production associated with Complexes I and III in heart, muscle, and lung mitochondria (Iqbal et al., 2001; Tang et al., 2001) and Complex II in liver mitochondria (Cawthon et al., 2001) obtained from broilers with pulmonary hypertension syndrome.

Inefficiencies of function may also be hypothesized to occur from insufficient activity or expression of respiratory chain proteins. Oxidation of respiratory chain proteins would decrease their activity and, in turn, the overall efficiency (coupling) of the respiratory chain. In addition, free radicals cause oxidant-mediated repression of mitochondrial transcription (Kristal et al., 1994) that exacerbates mitochondrial dysfunction by inhibiting synthesis of respiratory chain proteins (Kristal et al., 1997).

Thus, the first objective of the present study was to determine if differences in muscle mitochondrial function exist in broilers with different feed efficiencies within a single genetic line fed the same diet. The second objective was to assess amounts and sites of electron leak in muscle mitochondria in broilers with different feed efficiencies. The final objective was to determine respiratory chain complex activities and relationships of complex activities with feed efficiency in mitochondria isolated from broilers with different feed efficiencies.

MATERIALS AND METHODS

Birds and Management

Male broilers were selected from a group of 100 that were tested for feed efficiency in breeder male replacement stock.[4] At 6 wk, birds were individually housed in cages (51 × 51 × 61 cm)[5] at thermoneutral temperature (25 C; 15L:9D). Feed intake (from 6 to 7 wk) and 6- and 7-wk BW were determined to calculate feed efficiency (FE; g gain/g feed intake). From this group of birds, a 16 were identified that exhibited the lowest (n = 8) or highest (n = 8) FE within an initial group of 100 males. The birds were color-coded, transported to the University of Arkansas, and housed in similar cages and environmental conditions. Birds were provided access to water ad libitum and to the same diet provided during the feed efficiency trial (20.5% protein, 3,280 kcal/kg).

Sampling Procedure

After a 5-d acclimation, birds were randomly selected (one bird per day) from each group with group selection being alternated on successive days. Researchers at the University of Arkansas conducted these studies without knowledge of feed efficiency data until after the mitochondrial function studies were completed. After being weighed, each bird was killed with an overdose of sodium pentobarbital by i.v. injection into the wing vein. Portions of the breast muscle (pectoralis superficialis), leg muscle (quadriceps femoris) (Chamberlain, 1943), and liver were obtained for isolation of mitochondria (see below), and a portion of each tissue was immediately frozen in liquid nitrogen for biochemical analyses.

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Mitochondrial Isolation

Breast and leg muscle mitochondria were isolated according to Bhattacharya et al. (1991), with modifications. The tissues were excised rapidly, finely minced in isolation medium A (100 m*M* sucrose, 10 m*M* EDTA, 100 m*M* Tris-HCl, and 46 m*M* KCl, pH 7.4), and approximately 7 g of tissue was incubated at room temperature (25 C) in 14 mL of isolation medium A containing 20 mg % nagarase for 5 min. The minced tissue was homogenized in a Potter-Elvenhjem vessel with a Teflon pestle of 0.16-mm clearance[6] and was incubated for an additional 5 min on ice (4 C) with stirring. The homogenate (1,000 × *g* for 10 min) and resulting supernatant (10,000g for 15 min) were centrifuged to obtain the mitochondrial pellet that was resuspended and washed in 10 mL of isolation medium A plus 0.5% BSA (without nagarase). Mitochondria were pelleted by centrifugation (8,000 × *g* for 15 min) in incubation medium (230 m*M* mannitol, 70 m*M* sucrose, 20 m*M* Tris-HCl, and 5 m*M* KH_2PO_4, pH 7.4). The resulting pellet was resuspended in 2 mL of incubation medium and placed on ice for functional and respiratory inhibitor studies described below. Liver mitochondria were isolated according to Cawthon et al. (1999, 2001).

Mitochondrial Function

Mitochondrial function was determined according to Estabrook (1967). Oxygen consumption of mitochondria (expressed in nmol/min per mg protein) was measured polarographically with a Clark-type oxygen electrode in duplicate, 3 mL, thermostatically controlled chambers equipped with magnetic stirring[7] as recently described (Cawthon et al., 1999, 2001). All duplicate measurements were averaged and completed within 3 h of isolation. Aliquots (0.5 mL) of the muscle mitochondria were removed and added to the reaction vessel containing 1 mL of RCR reaction buffer (220 m*M* d-mannitol, 70 m*M* sucrose, 2 m*M* N-[2-hydroxyethylpiperizine]-N′-[2-ethanesulfonic acid] (HEPES), 3 m*M* KH_2PO_4; 5 μL of 1.5 m*M* rotenone, and 50 μL of 1 *M* succinate, pH 7.0). Substrates tested in this study were glutamate-malate (10:1 m*M*) and succinate (10 m*M*), which donate electrons to the respiratory chain at Complex I (NADH ubiquinone: oxidoreductase) and Complex II (succinate: ubiquinone oxidoreductase), respectively. Function in liver mitochondria was determined according to Cawthon et al. (1999) using succinate (10 m*M*) as an energy substrate.

Indices of muscle mitochondrial function were determined according to Estabrook (1967). After initial oxygen consumption (State 2 respiration) was monitored, State 3 (active) respiration was initiated following addition of 155 μM ADP (final concentration), followed by State 4 (resting) respiration when ADP levels became limiting. The acceptor control ratio (ACR) was calculated by dividing State 3 by State 2 respiration. The RCR (an index of respiratory chain coupling) was calculated as State 3 divided by State 4 respiration. The efficiency of ATP synthesis coupled to cell respiration, the ADP/O ratio, was determined by dividing the quantity of ADP added by the amount of oxygen consumed during State 3 respiration.

Mitochondrial H_2O_2 Production and Site-Specific Defects in Electron Transport

Generation of H_2O_2 was determined using the 2′,7′-dichlorofluorescin diacetate (DCFH-DA)[8] chemical probe using procedures by Iqbal et al. (2001) with modifications. Briefly, H_2O_2 was measured in 96-well microplates with a photofluorometric detector[9] at a sensitivity of 3 and excitation/emission wavelength at 480/530 nm, respectively. Reaction conditions for H_2O_2 measurement included addition of 0.1 mg of mitochondrial protein, 52 μM DCFH-DA, 64 μL H_2O_2 buffer containing 145 m*M* KCl, 30 m*M* HEPES, 5 m*M* KH_2PO_4, 3 m*M* $MgCl_2$, and 0.1 m*M* ethylene glycol-bis (β-aminoethylether)-N,N,N′,N′ tetraacetic acid (EGTA). Superoxide dismutase (10 U per well)[10] was added to each well of the microplate to convert all $O_2\bullet^-$ to H_2O_2. Mitochondria were provided with glutamate (8 m*M*) and malate (8 m*M*) as energy substrates that provide reducing equivalents to the electron transport chain at Complexes I. Activity remaining in wells with added catalase (225 Sigma units per well) was subtracted to account for fluorescence caused by factors other than H_2O_2 (Iqbal et al., 2001). The final volume in each well was 124 μL. The microplate was incubated at 37 C and read sequentially at 0, 10, and 30 min by a Cytoflour photofluorimeter. Values of H_2O_2 were calculated from a standard curve with known amounts of H_2O_2. Mitochondrial protein concentration was measured by the micro protein determination kit (# 610-A),[11] and values of H_2O_2 were expressed as nanomoles per minute per milligram of mitochondrial protein.

Generation of H_2O_2 in muscle mitochondria was monitored with and without chemical inhibitors that block electron transfer at specific sites in the respiratory chain as follows: rotenone (Complex I); 4,4,4-trifluoro-1-[2-thienyl]-1,3-butanedione (TTFA) and malonate (Complex II); myxothiazol (Complex III, Q cycle); and antimycin A (cytochrome b_{562} within Complex III). Final concentrations used were 10 μM rotenone, 13 μM myxothiazol, 8 μM TTFA, 13 μM antimycin A, and 7 μM malonate under the reaction conditions mentioned above. Appropriate controls were used for all wells of the microplate, e.g., blanks for mitochondria, all inhibitors, and catalase with both substrates and final values were corrected with these blanks.

[6]Thomas Scientific, Swedesboro, NJ.
[7]Yellow Springs Instrument Co. Inc., Yellow Springs, OH.
[8]Molecular Probes Inc., Eugene, OR.
[9]Cytofluor 2350 (Photofluorometric Detector), Millipore Corporation, Bedford, MA.
[10]Sigma Chemical Co., St. Louis, MO.
[11]SAS Institute Inc., Cary, NC.

TABLE 1. Growth performance data for broilers with low or high feed efficiency (FE)[1]

Variable	High FE (n = 6)	Low FE (n = 7)	*P*-value
6 wk BW (g)	2,390 ± 38	2,376 ± 10	0.702
7 wk BW (g)	3,324 ± 56	3,111 ± 66	0.043
Gain (g)	935 ± 42	735 ± 65	0.041
Feed (g)	1,134 ± 61	1,147 ± 85	0.911
FE (g gain/g feed)	0.83 ± 0.01	0.64 ± 0.01	<0.0001
FCR[2] (g feed/g gain)	1.21 ± 0.02	1.57 ± 0.03	<0.0001

[1]Values are mean ± SE of values shown in parentheses.

[2]Feed conversion ratio.

Respiratory Chain Complex Activities

Activities of Complex I (NADH ubiquinone:oxidoreductase) and Complex II (succinate: ubiquinone oxidoreductase) were assessed by ultraviolet spectrophotometry. Briefly, Complex I activity was measured by following the oxidation of NADH (Galante and Hatefi, 1978). Fifty microliters of mitochondria (~100 mg protein) was added to a solution containing 50 m*M* tris-HCl and 1.3 m*M* 2,6 dichloroindophenol (DCIP) in a final volume of 1 mL. The reaction was initiated with addition of 15 m*M* NADH. Absorbance at 600 nm was monitored for 10 min to follow the rate of oxidation of NADH and activity determined using an extinction coefficient of $\varepsilon = 21$ mM^{-1} cm^{-1}. Complex II activity was determined by following the reduction of DCIP by dihydroubiquinone-2 (Coenzyme Q_2) (Hatefi and Stiggall, 1978). Mitochondria (~100 μg protein) were added to a solution containing 74 μM DCIP and 50 μM Coenzyme Q_2. The reduction of DCIP was followed at 600 nm as a function of time until about 80% of the dye (DCIP) was bleached. Enzyme activity was calculated with an extinction coefficient of $\varepsilon = 21$ mM^{-1} cm^{-1}. Values for Complex I and II are expressed in units of activity per minute per milligram of mitochondrial protein.

Statistical Analyses

Data are presented as the mean ± SEM, and means were separated by *t*-tests. Regression analysis was accomplished using JMP In statistical analyses software.[11] A probability level of $P \leq 0.05$ was considered statistically significant.

RESULTS

Growth performance data are provided in Table 1. Successful mitochondrial function studies were conducted on seven and six birds in the low and high FE groups, respectively. Initial BW (at 6 wk) was not different, but the high FE birds were heavier at 7 wk due to faster growth rate, as there were no differences in feed intake ($P = 0.91$) between groups. Feed efficiency (g gain/g feed) was 0.64 ± 0.01 and 0.83 ± 0.01 for low and high FE groups, respectively (Table 1). Feed conversion ratios (FCR; g feed/g gain) for each group are also shown in Table 1. The dependency of body weight gain on feed intake is clearly indicated in Figure 1.

Functional Studies

There were no differences in breast muscle and leg muscle mitochondrial protein isolated from broilers with high and low FE; values were 3.2 ± 0.4 and 2.5 ± 0.3 mg/mL for high and low FE breast muscle mitochondria and 3.1 ± 0.4 and 2.6 ± 0.2 mg/mL for high and low FE leg muscle mitochondria, respectively. Values for respiration rates for high and low FE birds are provided in Table 2. There were no differences between high and low FE mitochondrial respiration for State 2 (prior to ADP addition), State 3 (active respiration in the presence of excess ADP), or State 4 (resting respiration when ADP becomes limiting) in muscle or liver mitochondria in this study. With glutamate-malate as an energy source, State 2 respiration was higher in leg muscle than in breast muscle in the high FE group. Leg muscle respiration rates (States 2, 3, and 4) were all greater in the high FE group than in breast muscle mitochondria when succinate was provided as an energy source. There were no differences in respiration rates between leg and breast muscle mitochondria in the low FE group when glutamate-malate or succinate was provided as an energy source. Muscle

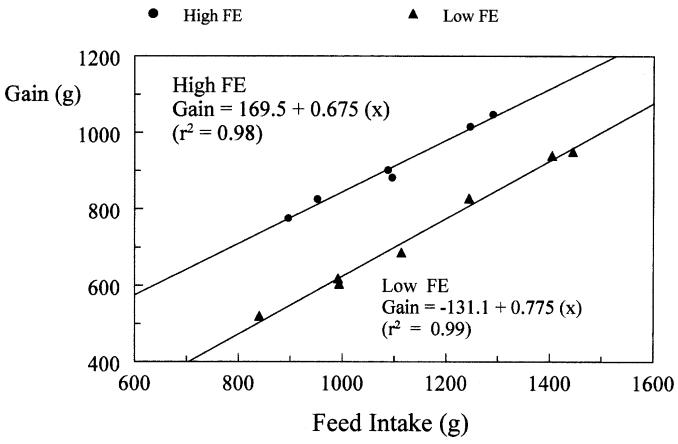

FIGURE 1. Relationships between total feed intake and body weight gain between 6 and 7 wk of age for male broiler breeders with high and low feed efficiency (FE). Regression equations shown were significant ($P < 0.05$).

TABLE 2. Mitochondrial oxygen consumption for States 2, 3, and 4 respiration rates in breast and leg muscle and liver mitochondria provided glutamate-malate or succinate as an energy substrates isolated from broiler males with high or low feed efficiency (FE)

		Glutamate-malate			Succinate		
Group	Tissue	State 2	State 3	State 4	State 2	State 3	State 4
		— (natoms O/min per mg protein) —			— (natoms O/min per mg protein) —		
High FE	Breast	18 ± 2[b]	129 ± 23	16 ± 3	43 ± 10[b]	87 ± 17[bc]	38 ± 9[b]
	Leg	24 ± 2[a]	132 ± 13	16 ± 1	65 ± 8[a]	135 ± 15[a]	60 ± 7[a]
	Liver	. . .	. . .	. . .	17 ± 1[c]	75 ± 5[c]	19 ± 1[c]
Low FE	Breast	22 ± 3	119 ± 17	20 ± 2	53 ± 9[ab]	109 ± 16[ab]	49 ± 8[ab]
	Leg	24 ± 2	112 ± 14	19 ± 2	76 ± 8[a]	146 ± 13[a]	69 ± 6[a]
	Liver	. . .	. . .	. . .	19 ± 1[b]	71 ± 6[b]	20 ± 1[b]

[a–c]Respiration values within a column with different letters are different ($P < 0.05$).

[1]Values represent the mean ± SE of n = 6 (High FE) and n = 7 (Low FE).

mitochondria provided succinate exhibited higher respiration rates compared to liver mitochondria in high and low FE birds.

When muscle mitochondria were provided glutamate-malate (which provides electrons to the transport chain at Complex I), the RCR (State 3/State 4) was higher ($P < 0.01$) in breast and leg muscle mitochondria in the high FE group (Figure 2A). These results indicate that electron transport was higher (more tightly coupled) in high FE than in low FE muscle mitochondria. Regression analysis revealed that breast mitochondria RCR values were highly correlated with FE (y = 11.3 (FE) – 1.20, $r^2 = 0.72$, $P < 0.001$)). Leg muscle mitochondria RCR values were also correlated with feed efficiency (y = 7.9 (FE) – 0.14, $r^2 = 0.37$, $P < 0.01$)). Marginally higher ACR values ($P < 0.06$) were also observed in high FE breast muscle mitochondria provided glutamate-malate. There were no differences in mitochondrial function in muscle mitochondria provided succinate between high and low FE birds (Figure 2B). These findings provide indirect evidence that functional differences (i.e., differences in electron transport chain coupling) between the two groups might be due to differences associated with electron transport within Complex I. There were also no differences in the ADP:O with either energy substrate, which indicated that there were no apparent differences in the ability of the leg and breast muscle to carry out oxidative phosphorylation between high and low FE birds. In the liver, high FE mitochondria provided succinate exhibited a higher ACR than did low FE mitochondria, but there were no differences in the RCR or ADP:O between groups (Figure 3).

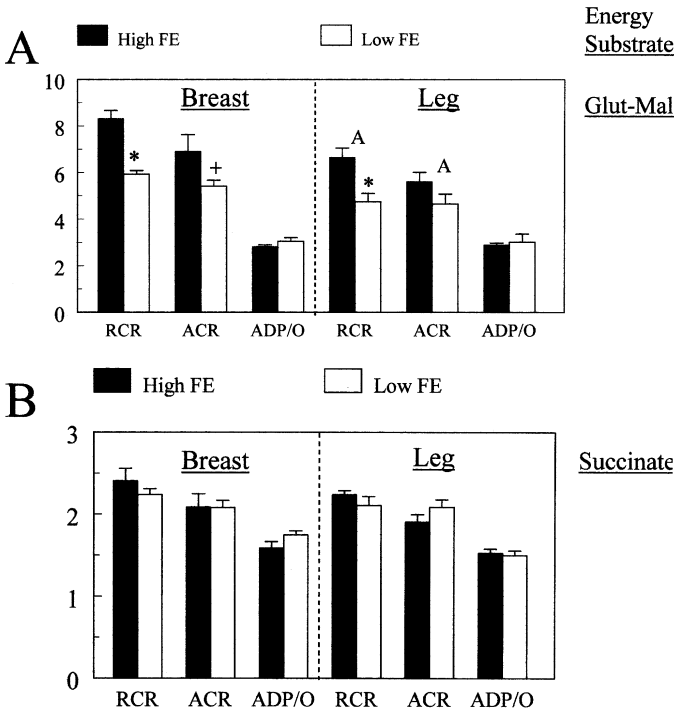

FIGURE 2. Mitochondrial function in breast and leg muscle isolated from broilers with high (shaded bars) or low (open bars) feed efficiency (FE). Functional measurements include the respiratory control ratio (RCR), the acceptor control ratio (ACR), and the ADP/O ratio for breast and leg muscle mitochondria provided A) glutamate-malate (Glut-Mal) or B) succinate as energy sources. Values represent the mean ± SE for high FE (n = 6) and low FE (n = 7). *Mean value for low FE is lower than high FE mitochondria ($P < 0.05$). +Mean value for low FE is lower than high FE mitochondria ($P < 0.06$). A main effect of leg muscle mitochondrial function is lower than breast muscle value ($P < 0.05$).

Determination of H_2O_2 Generation

To determine if the lower RCR values in Low FE muscle mitochondria might be associated with increased electron leak from the respiratory chain, H_2O_2 production in breast and leg muscle mitochondria was determined with and without various inhibitors of the electron transport chain. Basal H_2O_2 production (no inhibition) was greater ($P < 0.06$) in low FE than in high FE breast muscle mitochondria (Figure 4A). Inhibiting electron transport at Complex I with rotenone and Complex III (cytochrome B_{562}) with antimycin A raised H_2O_2 production in low FE, but not in high FE, breast muscle mitochondria. No differences were observed when electron transport was inhibited at Complex II (with malonate and TTFA) or the Q cycle of Complex III (with myxothiazol). There were no differences in H_2O_2 production between groups with any inhibitor treatment in leg muscle mitochondria. However, a rotenone-induced elevation ($P < 0.07$) observed in low FE, but not in high FE leg mitochondria, indicates that Complex I may be a potential site of electron leak in low FE leg muscle mitochondria.

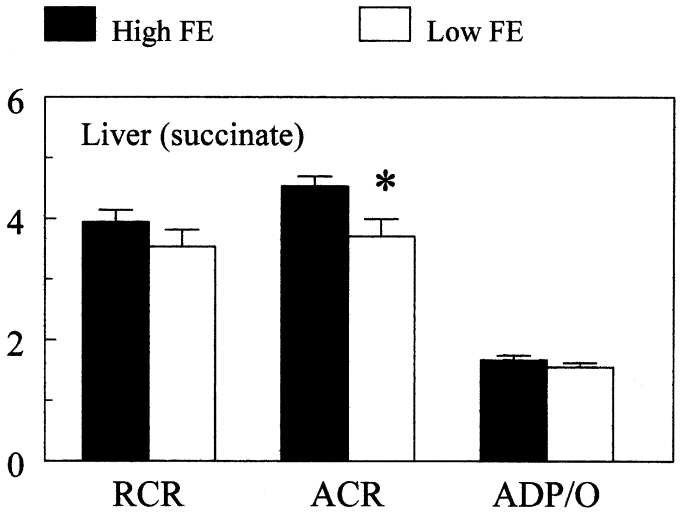

FIGURE 3. Mitochondrial function in liver isolated from broilers with high (shaded bars) or low (open bars) feed efficiency (FE). Functional measurements include the respiratory control ratio (RCR), the acceptor control ratio (ACR), and the ADP/O ratio for liver mitochondria provided succinate as an energy source. Values represent the mean ± SE for high FE (n = 6) and low FE (n = 7). *Mean value for low FE mitochondria is lower than high FE mitochondria ($P < 0.05$).

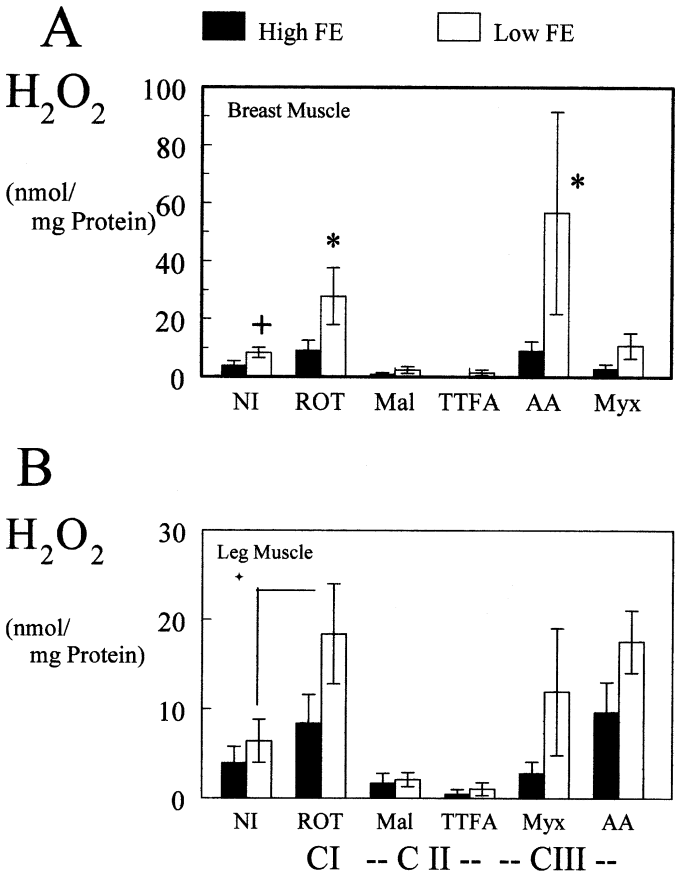

FIGURE 4. H_2O_2 production (nmol/min per mg mitochondrial protein [P]) in A) breast and B) leg muscle mitochondria obtained from broilers with high (shaded bars) and low (open bars) feed efficiency (FE). Mitochondria (provided glutamate as an energy substrate) were treated with no inhibitor (NI) or treated with rotenone (Rot), malonate (Mal), thenoyltrifluroacetone (TTFA), antimycin A (AA), and myxothiazol (Myx), which inhibit electron transport at Complexes (C) I, II, and III of the respiratory chain. Each bar represents the mean ± SE for high FE (n = 6) and low FE (n = 7). +Mean value in the Low FE group is higher than High FE ($P < 0.06$). *Within group treatment values are higher than no inhibitor (NI) value ($P < 0.05$). ◆Within group treatment value for rotenone is higher than no inhibitor (NI) value in leg muscle mitochondria ($P < 0.07$).

Respiratory Chain Complex Activities

Activities of NADH-linked (Complex I) and FADH-linked (Complex II) in breast and leg muscle mitochondria were lower in low FE than in high FE muscle mitochondria (Figure 5). Regression analysis revealed that activities of Complex I and II were significant ($P < 0.05$) and positively correlated (r^2 values ranging from 0.30 to 0.37) with feed efficiency for both muscle mitochondrial types (data not shown). Interestingly, when activities of Complexes I and II were combined and regressed with feed efficiency, this combined activity value improved the correlation coefficients ($r^2 = 0.41$ and 0.54), and the slopes depicting complex activity and feed efficiency relationships for each muscle were nearly parallel (Figure 6). The relationship in the Complex II to Complex I (CII:CI) activity ratio indicates more variation in relative activities of Complexes I and II was observed in low FE muscle mitochondria than was observed in high FE (Figure 7). The CII:CI ratio was significantly greater in low FE leg mitochondria (Figure 7, inset). These data suggest that a better balance in respiratory chain activities may be related to high feed efficiency as well.

DISCUSSION

Differences in rates of oxygen utilization between various breeds of chicken (Mukherejee et al., 1970; Dziewiecki and Kolataj, 1976; Brown et al., 1986) and swine (Dzapo and Wassmuth, 1983) have been previously reported. It has also been hypothesized that mitochondrial function may be part of the basis for heterosis that is observed in plants (McDaniel and Sarkissian, 1966; Srivastava, 1981), sheep and swine (Wolanis et al., 1980; Dzapo and Wassmuth, 1983), and chickens (Brown et al., 1986). However, the results of the present study are the first, to our knowledge, to provide evidence that mitochondrial function may be inextricably linked to feed efficiency within a single genetic line of broilers.

The importance of feed efficiency, rather than body weight gain, as the performance criteria for comparing the two groups is clearly shown in Figure 1. Muscle mitochondria from broiler males designated as having low FE exhibited lower RCR values, suggesting a decrease in respiratory chain coupling (Figure 2A), higher electron leak from the respiratory chain in breast muscle, (Figure 4A), and lower activities of Complexes I and II of the respiratory chain (Figure 5). It should be recognized that birds designated as low FE in this study (Table 1) would be superior in feed efficiency when compared to commercial broilers, even when accounting for any differences that would be expected to occur between laboratory and field conditions. Thus, mitochondrial function detected in the low and high FE groups in this study actually

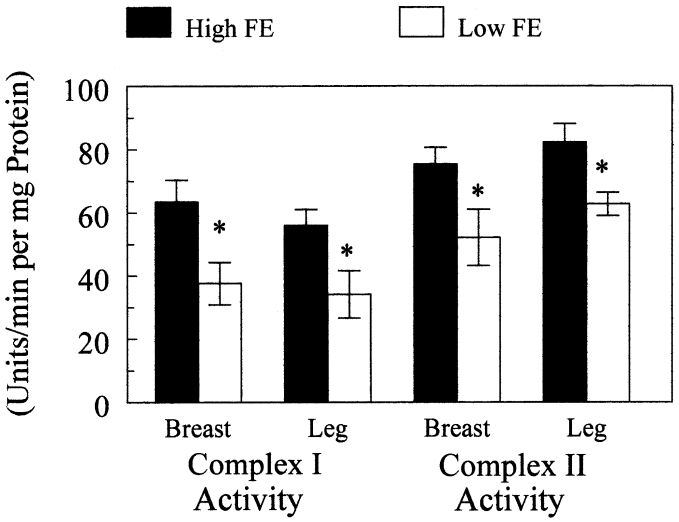

FIGURE 5. Activities (units/min per mg mitochondrial protein) of Complexes I and II in breast and leg muscle mitochondria obtained from broilers with high (shaded bars) and low (open bars) feed efficiency (FE). Each bar represents the mean ± SE for high FE (n = 6) and low FE (n = 7). *Low FE values are lower than High FE values ($P < 0.05$).

reflect differences within groups of very efficient birds. Differences that are even more dramatic might be obtained if a greater range of feed efficiencies had been examined. It should also be recognized that the results of the in vitro functional experiments studies provide only a single snapshot of what may be continuously occurring in vivo. Greater differences in mitochondrial function or radical production between low and high FE groups would likely be obtained over prolonged periods of time. Thus, accumulative effects of enhanced ROS production and lower respiratory chain coupling could be contributing to the phenotypic expression of feed efficiency between the groups of birds in this study over time.

Dietary-induced alterations in feed efficiency appear to have effects on mitochondrial function that may depend upon a variety of factors including tissue, species, and type of dietary alteration. For example, Toyomizu et al. (1992c) reported that decreased feed efficiency due to adding 2,4-dinitrophenol 400 mg/kg diet for broiler chicks had no affect on the ADP:O ratio in muscle and liver mitochondria. Reduced feed efficiency associated with feeding different sources of fat has no effect on liver mitochondrial function in mice (Toyomizu et al., 1992b) but lowers the RCR and ADP:O in White Plymouth Rock chicken heart mitochondria (Renner et al., 1979) and in liver mitochondria in rats (De Schrijver and Privett, 1984). Increasing the dietary protein to metabolizable energy from 25 to 61% reduces the ADP:O ratio but has no effect on the RCR or State 3 respiration in heart and liver mitochondria in Arbor Acre broiler chicks (Toyomizu et al., 1992a). Unlike the present study in which birds were provided the same diet, these studies all used different diets to alter feed efficiency. They do, however, provide insight into how chemical alterations of membranes or shifts in metabolism may affect mitochondrial function.

Breast muscle mitochondria obtained from low FE broilers exhibited elevated levels of H_2O_2 generation after inhibition of electron transfer with rotenone and antimycin A. These findings indicate that low FE mitochondria exhibit greater electron leak than high FE breast mitochondria, that this leak may be due to defects in electron

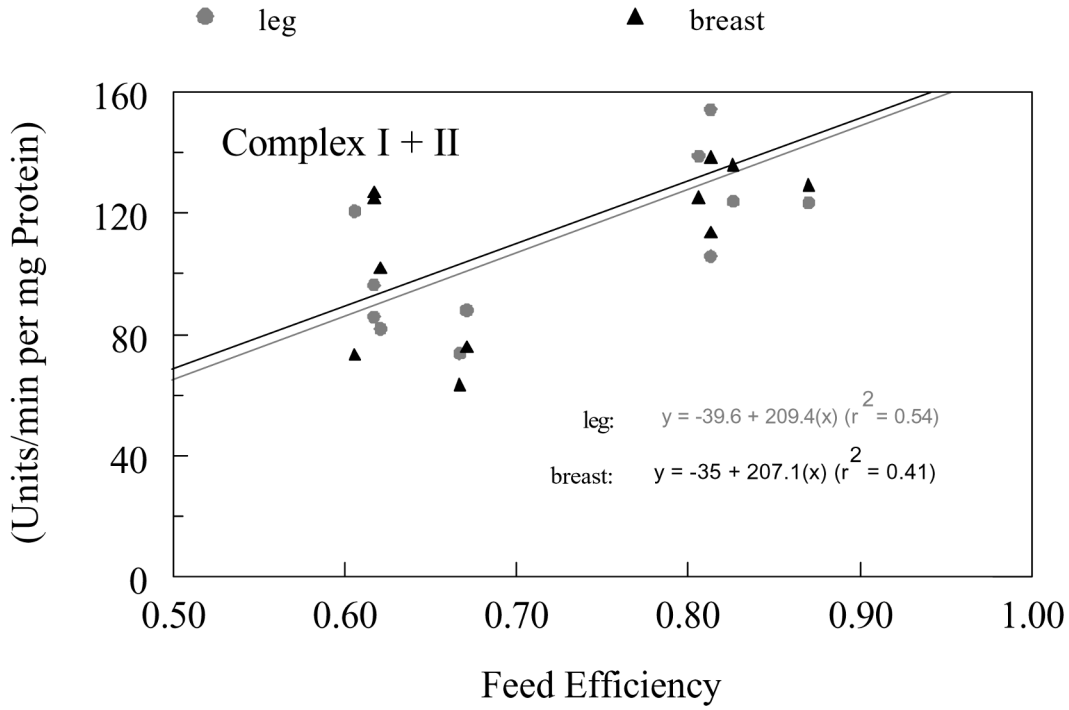

FIGURE 6. Relationships between feed efficiency and Complex I plus Complex II activity (units/min per mg mitochondrial protein) in breast (triangle) and leg (circle) muscle mitochondria. Regression equations shown for each relationship were significant ($P < 0.05$).

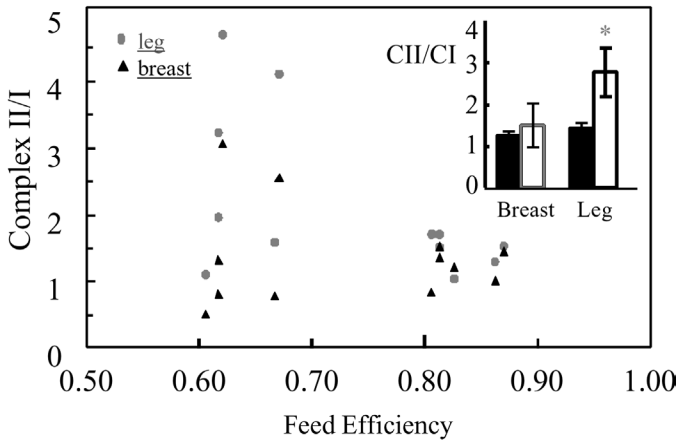

FIGURE 7. Relationships between feed efficiency and the Complex II to Complex I activity ratio (CII/CI) in breast and leg muscle mitochondria obtained from broilers with low and high FE.

transport within Complexes I and III (cytochrome b_{562}), and concur with previous findings indicating that electron leak from the respiratory chain occurs mainly within Complex I or III of the respiratory chain (Turrens and Boveris, 1980; Nohl et al., 1996; Herrero and Barja, 1998). Importantly, high FE muscle mitochondria did not exhibit significant increases in H_2O_2 production after inhibition of electron transport activity at Complexes I and III (Figure 4A and B). This finding may represent a key aspect in the understanding of the role of mitochondria in feed efficiency and suggests that mitochondria from high FE birds would exhibit very low amounts of electron leak in vivo. Increased radical production has also been observed at Complexes I and III in heart mitochondria in broilers with pulmonary hypertension syndrome (Tang et al., 2001). As mitochondrial ROS production was associated with mitochondrial dysfunction (e.g., lower RCR values) in several tissues obtained from broilers with pulmonary hypertension syndrome (Cawthon et al., 2001; Iqbal et al., 2001; Tang et al., 2001), higher amounts of oxygen radical production in low FE mitochondria could also contribute to the lower RCR values in mitochondria provided glutamate-malate (Complex I substrate) observed in the present study (Figure 2A).

The activities of Complexes I and II were lower in breast and leg muscle mitochondria obtained from broilers with low FE compared to activities in high FE muscle mitochondria (Figure 5). The expression of respiratory chain proteins is under dual genetic control by nuclear and mitochondrial DNA (Sue and Schon, 2000). The mitochondrial genome is found in every nucleated cell with 2 to 10 copies per mitochondrion, and up to 800 mitochondria can be present within a single cell (Robin and Wang, 1988). Mitochondrial DNA encodes 22 tRNA, 2 rRNA, and 13 proteins that are subunits of the various respiratory chain complexes (Anderson et al., 1981; Desjardins and Morais, 1990). Free radicals cause oxidant-mediated repression of mitochondrial transcription (Kristal et al., 1994) that exacerbates mitochondrial dysfunction by inhibiting synthesis of respiratory chain proteins (Kristal et al., 1997). The proximity of the respiratory chain to the relatively unprotected mitochondrial DNA and accessory proteins required for transcription makes mitochondrial transcription vulnerable to oxidative stress (Kristal et al., 1994). Seven of the 40 proteins that make up Complex I, the largest respiratory chain complex, are encoded by mitochondrial DNA (Anderson et al., 1981; Desjardins and Morais, 1990; Sue and Schon, 2000). Thus, one hypothesis to explain the lower respiratory complex activity in low FE breast muscle mitochondria (Figure 5) could be due to oxidative damage of mitochondrial DNA as a result of increased oxygen radical production (Figure 4A).

A second hypothesis to account for the lower activities of Complexes I and II could be that the increased ROS generation in low FE mitochondria cause direct oxidative damage to the respiratory chain proteins. However, activities of Complexes I and II were also lower in leg muscle mitochondria (Figure 5) that did not exhibit differences in radical production between groups with any inhibitor, although there was a marginal elevation in radical production following inhibition of Complex I activity with rotenone (Figure 4B). All four proteins associated with Complex II are encoded by nuclear DNA (Sue and Schon, 2000). Additionally, it was observed that the activity of glutathione peroxidase in whole breast muscle homogenate obtained from low FE birds was also lower when compared to values in muscle homogenate obtained from high FE broilers (unpublished observations).

Thus, a third hypothesis for the lower activity of Complexes I and II in low FE mitochondria could be due to an inherently lower expression of several key respiratory chain or antioxidant proteins in this group of birds. It is also possible that the lower respiratory chain complex activities in low FE could be a combination of oxidative damage to mitochondrial DNA and mitochondrial proteins combined with inherent differences in protein expression between low and high FE broilers.

To our knowledge, relative activities or variations in the ratio of the activities of Complexes I and II have not been previously reported with regard to comparing groups of animals in health or disease. Nevertheless, the CII:CI activity ratio provided additional insight regarding relationships between mitochondrial function and feed efficiency; low FE mitochondria exhibited a much greater variation in the CII:CI ratio than did high FE mitochondria (Figure 7). This finding is particularly interesting due to the fact that Complexes I and II accept electrons from NADH-linked and FADH-linked energy substrates. Thus, a more balanced activity ratio for Complexes I and II may be needed for efficient mitochondrial energy utilization and function, cell function, that in turn, may be required for transducing energy in feed to energy that can be used by the cell. This could be vitally important in ultimately contributing to greater feed efficiency in broilers.

To our knowledge, this report is also the first in which mitochondrial function in predominantly red (leg) and white (breast) muscle fibers have been researched in poultry. Electron microscopy revealed higher mitochondrial

content in red fibers than in white (Hoppeler et al., 1987), which may account for the higher aerobic capacity in red versus white muscle fibers in mammals (Baldwin et al., 1972). Jackman and Willis (1996) reported that the gracillis muscle (white fiber type) exhibited 50% of maximal activity of several inner mitochondrial membrane protein complexes compared to soleus (red fiber type) in rabbits, suggesting that there is roughly one-half the enzymatic protein of the respiratory chain in white muscle mitochondria. These results differ somewhat from the present study in chickens in which there was little difference in activities of Complexes I and II between breast and leg muscle mitochondria within the high or low FE group (Figure 5). The reason for the differences in results between the present study and that of Jackman and Willis (1996) is not apparent but could be due to species or could reflect differences in methodology or recovery of mitochondria.

In the present study, respiratory chain complex activities were measured by ultraviolet spectroscopy in isolated mitochondria after a single freeze-thaw procedure, whereas Jackman and Willis (1996) measured maximal respiration rates polarographically of respiratory chain components following repeated (five times) sonication and freeze-thaw procedures. However, using the same logic as Jackman and Willis (1996), the results of the complex activity measurements would suggest that low FE muscle mitochondria exhibit 20 to 40% lower expression of inner mitochondrial membrane proteins compared to high FE mitochondria (Figure 5). Caution should be used when equating enzyme activity with protein expression. Nonetheless, the fact that enzyme activity of mitochondrial and whole tissue (above) are lower in low FE birds suggests that the phenotypic expression of feed efficiency may be associated with differences expression of key proteins associated with mitochondrial function and antioxidant activity.

The higher RCR values in breast muscle compared to leg muscle mitochondria provided NADH-linked energy substrates (Figure 2A) concurs with findings in rabbit muscle mitochondria (Youlanda and Blanchaer, 1970). State 3 respiration rates with Complex I (NADH-linked substrates) were higher in red muscle than white muscle in the rabbit (Jackman and Willis, 1996). There were no differences in State 3 respiration between leg (red) and breast (white) muscle in low or high FE birds metabolizing glutamate-malate (NADH-linked substrate) (Table 2). However, respiration rates were greater in high FE leg muscle mitochondria than in breast muscle provided succinate, but differences in respiration rates were not observed between muscles in low FE mitochondria (Table 2). What role the difference in respiration rates between leg and breast muscle mitochondria in high FE birds, but not in low FE birds, contributes to mitochondrial function or feed efficiency is not apparent.

In summary, the results of this study provide evidence that mitochondrial function, respiratory chain activity, and electron leak are linked to feed efficiency in broiler breeder males identified as having low or high feed efficiency. Future studies are planned to determine if differences in respiratory chain complex activity in muscle between broilers with different feed efficiencies are due to genetic expression of these proteins, post-translational modification, or oxidative damage of proteins.

ACKNOWLEDGMENTS

The authors thank G. Huff, N. Rath, and D. Horlick (USDA-ARS, University of Arkansas) for assistance in the use of the Cytofluor 2350 used in the detection of 2′,7′-dichlorofluorescein (DCF) fluorescence. Appreciation is also extended to R. McNew (Agriculture Statistics Lab, University of Arkansas) for statistical design and consultation and to H. Brandenburger for technical editing. The research is supported in part by a grant from Cobb Vantress-Inc. (Siloam Springs, AR), a grant from USDA-NRI (2001-03443), and a visiting fellowship from the Peoples Republic of China (to Z. Tang). The research is published with approval of the Director of the Arkansas Agricultural Experiment Station.

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