CELL BIOLOGY SYMPOSIUM:
Feed efficiency: Mitochondrial function to global gene expression\(^1,2\)

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ABSTRACT: Understanding the cellular basis of feed efficiency (FE) is instrumental to helping poultry and livestock industries continue to provide high-quality protein for an increasingly crowded world. To understand relationships of FE and gene expression, global RNA transcription was investigated in breast muscle obtained from a male broiler line fed the same diet and individually phenotyped for FE. In these studies, RNA samples obtained from broilers that exhibited either high FE (0.65 ± 0.01) or low FE (0.46 ± 0.01) were analyzed with an Agilent 44K chicken oligoarray. A 1.3-fold cutoff in expression (30% difference between groups) resulted in 782 genes that were differentially expressed (\(P < 0.05\)) in muscle between the high- and low-FE phenotypes. Ingenuity Pathway Analysis, an online software program, was used to identify genes, gene networks, and pathways associated with the phenotypic expression of FE. The results indicate that the high-FE phenotype exhibited increased expression of genes associated with 1) signal transduction pathways, 2) anabolic activities, and 3) energy-sensing and energy coordination activities, all of which would likely be favorable to cell growth and development. In contrast, the low-FE broiler phenotype exhibited upregulation of genes 1) associated with actin-myosin filaments, cytoskeletal architecture, and muscle fibers and 2) stress-related or stress-responsive genes. Because the low-FE broiler phenotype exhibits greater oxidative stress, it would appear that the low-FE phenotype is the product of inherent gene expression that is modulated by oxidative stress. The results of these studies begin to provide a comprehensive picture of gene expression in muscle, a major organ of energy demand in an animal, associated with phenotypic expression of FE.

Key words: feed efficiency, global gene expression, mitochondria, muscle


INTRODUCTION

Feed efficiency (FE, G:F) remains an important genetic trait in livestock as costs of feed grain continue to rise worldwide. Feed costs in the United States have been accentuated by the severe drought conditions during the summer of 2012 (Nixon and Lowry, 2012). Feed efficiency is a complex multifaceted trait that is affected at many different levels ranging from the whole animal (e.g., behavioral differences) to environmental effects (e.g., temperature, nutrients) to differences at the cellular level. Skeletal muscle is a major organ system in an animal, and muscle development for human consumption as meat is a major “purpose” of commercial animal agriculture. Differences in skeletal muscle metabolism have been shown to be a major contributing factor for

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energy metabolism (e.g., Zurlo et al., 1990). Proton leak, which is just one component of mitochondrial function affecting ATP synthesis, has been reported to contribute to as much as 50% of oxygen usage in perfused muscle (Brand, 1990; Rolfe and Brand, 1996). Thus, investigations of cell physiology and gene expression within a major skeletal muscle, such as breast muscle in poultry, may help in “dissection” of critical components of cellular efficiency that would enhance understanding of overall FE in an animal.

An initial study in broilers provided a link between mitochondrial function and the phenotypic expression of FE (Bottje et al., 2002). Broilers in this study were from the same genetic line, fed the same diet, and individually phenotyped for FE. The animals were held in a thermoneutral environment, fed the same diet, and housed in individual cages that would take behavioral, dietary, and environmental factors out of the FE equation. Using a similar paradigm, considerable information has been reported regarding relationships of FE with mitochondrial function, biochemistry, protein expression, and gene expression in broilers (Iqbal et al., 2004, 2005; Ojano-Dirain et al., 2004, 2005a,b; 2007a,b,c; Lassiter et al., 2006; Bottje et al., 2009; Tinsley et al., 2010), rats and mice (Lutz and Stahly, 2003; Nisoli et al., 2003), cattle (Kolath et al., 2006; Lancaster et al., 2007; Kelly et al., 2011; Alam et al., 2012), trout (Eya et al., 2011), catfish (Eya et al., 2012), sheep (Sharifabadi et al., 2012), and swine (Gabler et al., 2012).

In the low-FE broiler phenotype, at least 2 processes were identified that would contribute to mitochondrial inefficiency. Site-specific defects in electron transport with greater rates of mitochondrial reactive oxygen species (ROS) generation was likely responsible for greater oxidized protein levels in the low-FE phenotype (Bottje et al., 2006; Bottje and Carstens, 2009). Repair of damaged proteins is energetically expensive because considerable energy is expended in both protein synthesis and in ubiquitination and hydrolysis of damaged proteins (Ciechanover, 1998; Lecker et al., 2006). Because ROS also function as secondary messengers and signal transducers (Yu, 1994; Kristal et al., 1997), increased mitochondrial ROS may be responsible for differences in gene and protein expression in the low-FE phenotype.

A second mitochondrial inefficiency in the low-FE phenotype is proton leak (Bottje et al., 2009). Proton leak is the movement of protons across the inner mitochondrial membrane at sites other than through ATP synthase (Brand, 1990). Proton leak is responsible for up to 50% of basal oxygen consumption rate in mitochondria and is due to factors such as membrane and lipid characteristics, expression of uncoupling proteins, and intrinsic transport proteins (e.g., adenine nucleotide translocator; aspartate-glutamate transporter; Brown and Brand, 1991; Brookes et al., 1997, 1998; Talbot et al., 2003, 2004; Brand et al., 2005). Proton leak is an energetically wasteful process but serves a vital homeostatic function by attenuating mitochondrial ROS production via a superoxide-mediated stimulation of uncoupling protein activity (Skulachev, 1996, 1997; Echtay et al., 2002a,b).

In the low-FE phenotype broilers, avian uncoupling protein mRNA transcription in muscle was greater ($P < 0.07$) than in the high-FE phenotype (Ojano-Dirain et al., 2007b). Furthermore, in a comprehensive investigation of proton leak kinetics, proton leak rates were greater than or equal to (but never less than) proton leak in the high-FE phenotype (Bottje et al., 2009).

From the information discussed above, it is logical to assume that key genes associated with mitochondria function, activities, or development would be differentially expressed between the high- and low-FE phenotypes. It is also reasonable to hypothesize that gene expression in the low-FE phenotype could be altered with respect to the high-FE phenotype because of increased mitochondrial ROS production. Finally, because FE is a complex trait, it is likely that the phenotypic expression of FE is the result of large differences in expression of a few genes combined with small differences in expression of many genes that may or may not have a direct relationship with mitochondrial function. The remainder of this review will focus primarily on recent global gene expression studies in broilers.

Global Gene Expression and Feed Efficiency in Broilers

Global gene expression analysis was conducted on breast muscle obtained from high- and low-FE phenotype broilers (Kong et al., 2011; Bottje et al., 2012). Details regarding microarray analysis, data collection, bioinformatics, and validation of the 44K chicken oligoarray are described in those papers. Of the 44,000 gene sequences, 4,011 were differentially expressed ($P < 0.05$) by 15% (1.15-fold) or more and 782 were differentially expressed ($P < 0.01$) by at least 30% (1.30-fold) or more between the high- and low-FE broiler phenotypes. In these studies, a Web-based software program (i.e., Ingenuity Pathway Analysis, IPA; http://apps.ingenuity.com) was used to facilitate interpretation and understanding of gene networks and pathways associated with FE.

Top Differentially Expressed Genes

The top 10 differentially expressed (upregulated) genes in the high-FE phenotypes are presented in Table 1 (Kong et al., 2011). Using a build function of IPA, 8 additional upregulated genes in the high-FE phenotype and 13 additional upregulated genes in the low-FE phenotype were identified (Fig. 1; Kong et al., 2011).
Genes above the diagonal line in Fig. 1 depict those that were upregulated in the low-FE phenotype (downregulated in high FE) and those below the diagonal line represent genes that were upregulated in the high-FE phenotype (downregulated in low FE).

A metaplasia picture begins to emerge in Fig. 1 indicating that the high-FE phenotype is characterized by downregulation of 1) genes associated with major histocompatibility complex cell recognition, 2) stress-related heat shock protein genes (Hsp7 and HSPB7), and 3) several genes associated with muscle fibers or cytoskeletal architecture [e.g., troponin 1, α actinin, tropomyosin, Ca$^{2+}$–Mg$^{2+}$ adenosine 5’monophosphatase (ATPase), myosin, and myosin light chain]. Downregulation of genes involved in muscle organization, contraction, and/or cytoskeletal architecture may translate into greater cellular efficiency and implies that a less organized cytoskeletal architecture may be integral to the high-FE phenotype. Genes for several platelet derived growth factors (PDGF; e.g., PDGF-BB, PDGFB, PDGF, PDGF-DD, and PDGF-D) were also upregulated in the low-FE phenotype. Ye et al. (1996) reported that the BB and AB isoforms of PDGF enhanced satellite cell and embryonic turkey myoblast proliferation. Because cell proliferation consumes energy, the low-FE phenotype may spend more energy without necessarily producing more overall growth compared with the high-FE phenotype.

Upregulation of several genes associated with anabolic functions was observed in the high-FE phenotype (Fig. 1, below diagonal line). These upregulated genes include 1) carbonic anhydrases 1 and 4 and genes with scaffolding activity, including aldolase, 2) genes associated with purine and pyrimidine biosynthesis [phosphoribosyl pyrophosphate synthetase 1 and 2 (PRPS1 and PRPS2), nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide pyrophosphorylase (NAMS)], 3) protein packaging genes (golgin B1, GOLGB1), 4) genes involved in prevention or delay of apoptosis [anti-apoptotic protease activating factor 1 (APIP)], and 5) modulation of gene transcription domain bifurcated histidine N-methyl

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**Table 1.** Top upregulated and downregulated genes in the high feed efficiency (FE) phenotype as determined by Ingenuity Pathway Analysis (http://apps.ingenuity.com)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Entrez gene name</th>
<th>Microarray (n = 4)</th>
<th>2-ΔΔCt (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Top 10 upregulated genes in high FE (downregulated in low FE)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APIP</td>
<td>APAF1 interacting protein</td>
<td>3.3 ± 0.1</td>
<td>2.0 ± 0.4*</td>
</tr>
<tr>
<td>CA4</td>
<td>Carbonic anhydrase</td>
<td>2.1 ± 0.1</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>DCUN1D5</td>
<td>Defective in cullin neddylation 1 domain containing 5</td>
<td>2.1 ± 0.1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>TMEM163</td>
<td>Transmembrane protein 163</td>
<td>2.0 ± 0.1</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>C1QTNF3</td>
<td>C1q and tumor necrosis factor related protein</td>
<td>1.9 ± 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>GSTZ1</td>
<td>Glutathione transferase zeta 1</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>ALDOB</td>
<td>Aldolase B, fructose-biphosphate</td>
<td>1.8 ± 0.1</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>PRPS2</td>
<td>Phosphoribosyl pyrophosphate synthetase 2</td>
<td>1.8 ± 0.1</td>
<td>3.0 ± 0.2*</td>
</tr>
<tr>
<td>GOLGB1</td>
<td>Golgin B1, golgi integral membrane protein</td>
<td>1.8 ± 0.2</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>SETDB1</td>
<td>SET domain, bifurcated 1</td>
<td>1.8 ± 0.1</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td><strong>Top 10 downregulated genes in high FE (upregulated in low FE)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSRPS3</td>
<td>Cysteine and glycine-rich protein 3 (myogenesis)</td>
<td>6.3 ± 0.5</td>
<td>3.6 ± 1.0*</td>
</tr>
<tr>
<td>MYH6</td>
<td>Myosin, heavy chain 6, cardiac muscle, alpha</td>
<td>5.5 ± 0.4</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>MYOZ2</td>
<td>Myozenin 2 protein binding</td>
<td>3.3 ± 0.4</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>LMOD2</td>
<td>Leiomodin 2 (cardiac)</td>
<td>3.1 ± 0.3</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>TNNI1</td>
<td>Troponin I type 1 (skeletal slow)</td>
<td>2.9 ± 0.2</td>
<td>1.9 ± 0.4*</td>
</tr>
<tr>
<td>MYL10</td>
<td>Myosin light chain 10</td>
<td>2.9 ± 0.3</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>ADPRHL1</td>
<td>ADP-ribosehydrolase like 1</td>
<td>2.7 ± 0.3</td>
<td>5.8 ± 1.2*</td>
</tr>
<tr>
<td>TMOD1</td>
<td>Tropomodulin 1</td>
<td>2.5 ± 0.1</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>Major histocompatibility complex, class II, DR B1</td>
<td>2.4 ± 0.3</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>HSPB7</td>
<td>Heat shock 27kDa protein family, member 7</td>
<td>2.4 ± 0.2</td>
<td>1.9 ± 0.6</td>
</tr>
</tbody>
</table>

*The expression of these genes (fold upregulation) is shown for values obtained by microarray and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) methods (2-ΔΔCt). Values for fold difference in expression represent the mean ± SE of observations number of indicated Data are from Kong et al. (2011).

*The qRT-PCR values (2-ΔΔCt) are significantly different from microarray values (P < 0.05).
transf erase (SETDB1)]. (Note: The SET domain was originally identified as part of a larger conserved region that is present in the Drosophila trithorax protein and was subsequently identified in the Drosophila Su(var)3-9 and ‘Enhancer of zeste’ proteins, from which the acronym SET is derived (Jenuwein et al. 1998; Rea et al., 2000).

**Focus Genes in Phenotypic Expression of Feed Efficiency**

Relatively small differences in expression of key regulatory genes (e.g., kinases) could make major contributions to a particular FE phenotype. With this in mind, a group of focus genes was identified (Bottje et al., 2012) and is listed in Table 2. To draw attention to genes associated with normal cell function and away from abnormal conditions (e.g., cancer, disease), a subset of 263 genes containing 144 differentially expressed genes (46 upregulated and 98 downregulated) was derived from genes associated with the top 5 activities derived from the IPA program categorized under 1) molecular and cellular functions and 2) physiological system development and function. Next, 130 out of 260 possible canonical pathways in the IPA program that were not obviously disease or tissue specific were selected. Each canonical pathway was projected onto the 144 differentially expressed genes, and the number of times genes were “focused” by the canonical pathways was determined. Focus genes appeared in at least 6 of 130 pathways (5%; Table 2).

All upregulated focus genes in breast muscle of high-FE phenotype broilers were associated with either major signal transduction pathways and cascade mechanisms or involved in sensing of energy status and regulating energy production in the cell. This contrasts markedly with focus genes in the low-FE phenotype in which only 4 were directly linked to cell signaling cascade mechanisms [3 G protein-coupled receptor (GPCR) genes and serum/glucocorticoid-inducible Ser/Thr protein kinase (SGK1)]. Five focus genes in the low-FE phenotype were associated with cytoskeletal architecture (or actin-myosin filaments).

Nine upregulated focus genes in the high-FE broiler phenotype (Table 2) were associated with protein A (PKA) cascade mechanisms and the Jnk [c-Jun NH2-terminal kinase] pathway. After phosphorylating downstream targets, increased transcription and translation could enhance cell growth, proliferation, and differentiation. Evidence of phosphoinositol 3-kinase (PI3K) involvement in cell growth is well known (e.g., Leevers et al., 1996). Furthermore, greater upregulation of these genes could tip the balance from increasing to decreasing energetic efficiency. Extracellular-initiated PKA cascade mechanisms are mediated by binding of chemokines to G protein-coupled receptors or via the Jnk pathway in which growth factors bind to receptors (receptor tyrosine kinases, RTK; Table 2; Marone et al., 2008). Receptor binding activates PI3K and its activation would be accentuated by upregulation of 2 regulatory subunits (p85 and PIK3R1) in the high-FE phenotype. Whereas GPCR comprise the largest group of transmembrane signal transduction receptors (Roush, 1996; Pitcher et al., 1998; Fredriksson and Schioth, 2005; Ito et al., 2009), only GPR155 was upregulated in high FE (Table 2).

Phosphorylation of rat sarcoma GTPase (Ras), PKA, and mitogen-activated protein kinase kinase 4 (MAP2K4) activates Jnk. Translocation of Jnk into the nucleus, with c-fos- and c-Jun-mediated transcription and translation activities, would enhance cell growth.

### Table 2. Up- and downregulated focus genes in breast muscle of the high feed efficiency (FE) broiler phenotype

<table>
<thead>
<tr>
<th>Gene abbreviation</th>
<th>Gene name</th>
<th>Fold change (FC) exp1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upregulated focus genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3K</td>
<td>1-phosphatidylinositol 3-kinase regulatory subunit</td>
<td>1.31</td>
</tr>
<tr>
<td>PI3KR1/PI3K</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun NH2-terminal kinase</td>
<td>1.31</td>
</tr>
<tr>
<td>Sapk</td>
<td>Stress responsive protein kinase (synonym, Jnk)</td>
<td>1.30</td>
</tr>
<tr>
<td>p85(pi3r)</td>
<td>PI3K p85 subunit</td>
<td>1.31</td>
</tr>
<tr>
<td>Ras</td>
<td>Rat sarcoma proteins</td>
<td>1.30</td>
</tr>
<tr>
<td>MAP2K4</td>
<td>Mitogen-activated protein kinase</td>
<td>1.35</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
<td>1.31</td>
</tr>
<tr>
<td>PRKAr2</td>
<td>AMP-activated protein kinase gamma 2</td>
<td>1.36</td>
</tr>
<tr>
<td>ALDHIA3</td>
<td>Aldehyde dehydrogenase 1 family member A3</td>
<td>1.39</td>
</tr>
<tr>
<td>AMPK</td>
<td>AMP activated kinase</td>
<td>1.31</td>
</tr>
<tr>
<td>GNB1L(Gβ2)</td>
<td>Guanine nucleotide binding protein beta</td>
<td>1.52</td>
</tr>
<tr>
<td>PP2A</td>
<td>Protein phosphatase 2A</td>
<td>1.31</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor (GPR155)</td>
<td>1.60</td>
</tr>
<tr>
<td><strong>Downregulated focus genes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptors (C3AR1, RGR, P2RY13)</td>
<td>1.39</td>
</tr>
<tr>
<td>Myosin</td>
<td>Myosin</td>
<td>1.51</td>
</tr>
<tr>
<td>PDGF</td>
<td>Plate derived growth factor</td>
<td>1.45</td>
</tr>
<tr>
<td>MYL2A (MLC)</td>
<td>myosin light chain 12A, regulatory</td>
<td>1.52</td>
</tr>
<tr>
<td>Factin</td>
<td>Filamentous actin</td>
<td>1.31</td>
</tr>
<tr>
<td>HADHA</td>
<td>Mitochondrial long chain enoyl CoA hydratase</td>
<td>1.44</td>
</tr>
<tr>
<td>ACTA2</td>
<td>Smooth muscle alpha actin</td>
<td>1.42</td>
</tr>
<tr>
<td>Actin</td>
<td>G-actin</td>
<td>1.31</td>
</tr>
<tr>
<td>PDGFB</td>
<td>Plate derived growth factor beta</td>
<td>1.31</td>
</tr>
<tr>
<td>SOCS3</td>
<td>Suppressor of cytokine signaling 3</td>
<td>1.30</td>
</tr>
<tr>
<td>HSP90AB1</td>
<td>Heat shock protein 90 kDa alpha (cytosolic)</td>
<td>1.61</td>
</tr>
<tr>
<td>SGK1</td>
<td>Serine/threonine-protein kinase</td>
<td>1.55</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase (Mn-SOD, Cu-Zn-SOD)</td>
<td>1.58</td>
</tr>
</tbody>
</table>

1All were differentially expressed (P < 0.05) at either Fold change (FC) shown. Data are from Bottje et al. (2012).
Additional Jnk activation occurs via the retinoic acid (RA) and retinoid X receptor (RAR-RXR) pathway. All trans-RA regulates gene expression during embryonic development (Hoffman and Eichele, 1994; Maden, 1994; Noy, 2000). Upregulation of retinoic acid binding protein (RBP)-7, that facilitates retinol translocation, was also observed in the high-FE phenotype (Bottje et al., 2012). Conversion of retinol to RA is catalyzed by an aldehyde dehydrogenase (ALDH1A3), that was also upregulated in the high-FE phenotype (Table 2). Thus, RBP7 and ALDH1A3 may enhance growth and proliferation of muscle cells, possibly early in embryonic development, and contribute to the phenotypic expression of high FE through stimulation of the Jnk pathway.

The first documented reversible biochemical reactions involved phosphorylation of enzymes (Nolan et al., 1964) and are now well known to be very important in regulating numerous biochemical pathways in the cell. Protein phosphatase 2A (PP2A) regulates kinase phosphorylation in the PKA cascade (Janssens and Goris, 2001; Janssens et al., 2005; Eichhorn et al., 2009). Increased PP2A in the high-FE phenotype could represent a particularly critical gene, functioning to balance signal transduction and kinase cascade reactions with cell bioenergetics to optimize growth and development.

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) occupies a central and critical role in adjusting ATP production to match cell needs. Both AMPK and AMPK γ 2 (PRKAG2) mRNA were upregulated in the high-FE phenotype muscle. Expression of AMPK is stimulated by an increase in the AMP to ATP ratio (i.e., energy demand). Increased AMPK activity increases effective ATP levels by decreasing energy use and stimulating mitochondrial biogenesis (Zhou et al., 2001; Hardie et al., 2003; Carling, 2004; Hardie, 2007; Cantó and Auwerx, 2009). Proszkowiec-Weglarz and Richards (2009) demonstrated the functionality of the AMPK pathway in chick embryos and hypothesized that it could be particularly important in the transition from embryo to perinatal existence. A tonic upregulation of AMPK in the high-FE phenotype may be particularly instrumental in coordinating energy production efficiency. Food intake and energy balance regulation in the hypothalamus is mediated in part by AMPK activity (Minokoshi et al., 2004). Once phosphorylated, AMPK activates enzymes involved in carbohydrate, lipid, and protein metabolism (Kemp et al., 2003; Hardie, 2005, 2007).

In general, AMPK increases the ATP pool by reducing ATP-utilizing pathways (e.g., fatty acid biosynthesis) and increasing ATP-generating pathways (e.g., fatty acid oxidation, glycolysis). Whereas AMPK stimulates glucose uptake and glycolysis in muscle (Zhou et al., 2001; Almeida et al., 2004), AMPK inhibition prevented glycolysis when oxidative phosphorylation was uncoupled in tumor cells (Wu et al., 2007). Although AMPK can inhibit a critical step in the protein kinase A/mammalian target of rapamycin (i.e., Akt/mTOR) pathway leading to protein synthesis by inhibiting Rheb-mediated activation of mTOR complex 1 (mTORC1)(Bodine et al., 2001, Aoki et al., 2006; Schiaffino and Mammucari, 2011; Adegoke et al., 2012), we have evidence of upregulation of a large number of genes both upstream and downstream to where AMPK would exert its effect, which would likely offset any inhibition of protein synthesis in the high-FE phenotype (W. Bottje and B.-W. Kong, unpublished data). Thus, the activity of AMPK could be essential for maximizing cellular energetic efficiency.

In addition to differentially expressed focus genes, 7 of 41 genes in complex I were upregulated in the high-FE phenotype (Bottje et al., 2012). Complex I (NADH ubiquinone:oxidoreductase or NADH dehydrogenase) is the largest multiprotein complex of the respiratory chain (Lehninger et al., 1993). The NADH-linked energy substrates provide reducing equivalents to complex I for mitochondrial oxidative phosphorylation. Upregulation of these complex I genes is consistent with findings of greater complex I activities in high-FE phenotypes (Bottje et al., 2002; Bottje and Carstens, 2009; Sharifabadi et al., 2012). It is possible that increased expression of complex I was due to greater AMPK expression in the high-FE phenotype.

More evidence of oxidative stress in the low-FE phenotype is implicated by upregulation of stress responsive genes, including 1) SGK1, 2) corticotropin-releasing hormone (CRH), 3) several heat shock proteins (HSP) and heat shock transcription factors, and 4) superoxide dismutase (SOD). Proline-rich regulatory subunit 3A (SOD; Kong et al., 2011; Bottje et al., 2012). Heat shock proteins are ubiquitously expressed, induced by oxidative stress, and act as molecular chaperones to stabilize proteins and cytoarchitecture (Welch, 1992). Thus, increased HSP expression might be a response to increased mitochondrial ROS in the low-FE phenotype and could act to stabilize oxidatively damaged proteins that are apparently pervasive in low FE (Bottje and Carstens, 2009).

Increased expression of Mn-SOD and Cu-Zn-SOD would convey antioxidant protection in the mitochondria and cytosol, respectively. Because many antioxidants are induced by oxidative stress (Yu, 1994), it is not surprising that SOD was upregulated in the low-FE phenotype that exhibited increased mitochondrial ROS production in muscle, intestines, and liver (Bottje et al., 2002; Ojano-Dirain et al., 2004; Iqbal et al., 2005).

Although CRH was initially discovered in the hypothalamic portal system and determined to be a ubiquitous neurotransmitter (De Souza et al., 1985), de novo CRH expression was observed in several extrahypothalamic tissues (e.g., skin, uterus, immune system, and gastroin-
gestational tract; Slominski et al., 2001). Endogenous CRH expression was increased in breast muscle of the low-FE phenotype (Bottje et al., 2012). Direct CRH-mediated thermogenesis in muscle, by substrate cycling between lipogenesis and lipid oxidation (Solinas et al., 2006), indicates that CRH may play a role in energetic inefficiency in the low-FE phenotype. Finally, increased expression of S6K1 may also be in response to increased ROS in the low-FE broiler phenotype because it is upregulated by dexamethasone and H2O2 (Webster et al., 1993a,b; Leong et al., 2003).

A general upregulation of several cytoskeletal- or cytoarchitecture-related genes is apparent in the low-FE phenotype (Kong et al., 2011; Bottje et al., 2012). The gene exhibiting the greatest differential expression (6.3-fold in the low-FE phenotype), cysteine- and glycine-rich protein 3 (CSRP3, or muscle LIM protein, MLP), (see Zheng and Zhao, 2007 for a review of MLP) is an essential nuclear regulator of myogenic differentiation, organization, and maintenance of contractile machinery in skeletal and heart muscle (Arber et al., 1994, 1997; Kong et al., 1997; Louis et al., 1997). The ability of CSRP3 to regulate myogenesis is particularly interesting because it is expressed in both the cytosol and nucleus (Flick and Konieczny, 2000). Possibly, CSRP3 acts as a scaffolding protein because MLP-deficient mice exhibited decreased cytoskeletal architecture organization (Arber et al., 1997). Insufficiency of CSRP3 also led to a local loss of mitochondria and function, by disrupting mitochondrial-cytoskeletal interactions impairing energy sensing and balance (van den Bosch et al., 2005). A 3-fold upregulation in CSRP3 in undernourished cattle may counteract muscle atrophy by stimulating myogenic differentiation (Lehnert et al., 2006). Thus, increased CSRP3 expression along with several cytoskeletal-related genes (e.g., actin, troponin, and myosin) may be a response to increased protein oxidation in the low-FE phenotype (Kong et al., 2011). This also implies that energy directed toward cytoskeletal organization may contribute to the phenotypic expression of low FE, a hypothesis that is supported by downregulation of 2 ATPase enzymes in the high-FE phenotype (Kong et al., 2011).

Our findings appear to have similarities to those reported by Zheng et al. (2009), who found that several muscle-related genes (e.g., troponin 1, myosin light chain, and myosin heavy chain) were downregulated in broilers compared with layers. In addition, fibroblast growth factor 1, fibroblast growth factor receptor 2, and CSRP3 genes associated with muscle cell hypertrophy and satellite cell proliferation (Flick and Konieczny, 2000) were also downregulated in broilers (Zheng et al., 2009). Expression of fibroblast growth factor receptor 2 was 52% lower in the low-FE phenotype. As broilers obviously grow faster with greater muscle development than leghorns, downregulation of these genes clearly has no adverse effect on BW gain or muscle development. Thus, downregulation of muscle fiber- and cytoskeletal-related genes, combined with upregulation of signal transduction pathways, appears to play a significant role in the development of the high-FE broiler phenotype.

**Upstream Regulators and Mitochondrial Biogenesis**

Mitochondrial biogenesis refers to the ability of cells to increase mitochondrial numbers in response to increased energy demand. Hoppins et al. (2007) provides a detailed overview of dynamin proteins that bind to mitochondria to initiate a series of events leading to mitochondrial fission. Peroxisome proliferating factor peroxisome proliferator-activated receptor γ coactivator-1 α (PGC1-α) has been named the master regulator of mitochondrial biogenesis by stimulating expression of nuclear respiratory factor 1 and mitochondrial transcription factor A (Nisoli et al., 2003). We observed increased PPAR-γ expression in breast muscle and expression of both PPAR-γ and PGC1-α in duodenal tissue (Ojano-Dirain et al., 2007c). In yearling Limousin × Friesian beef heifers individually phenotyped for residual feed intake, Kelly et al. (2011) reported a negative correlation between RFI (RFI) and PGC1-α (r2 = −0.78, P < 0.01) and for PPAR-γ (r2 = −0.48, P < 0.1). In the microarray data set from Kong et al. (2011), PPAR-γ was not differentially expressed, but a gene designated as “similar to PGC1-α” that was not recognized by the IPA program was greater (P < 0.01) in the high-FE phenotype muscle with a fold differential expression of 1.24 (W. Bottje and B.-W. Kong, unpublished data). These findings provide additional evidence that mitochondrial biogenesis was enhanced in breast muscle of the high-FE broiler phenotype.

**Summary and Conclusions**

Figures 2, 3, and 4 will be used to summarize the previous discussion. Figure 2A starts with the role that upregulation of AMPK and PRKAG2 would have on the flow of substrate via glycolysis into the Kreb’s cycle and shows that the combined upregulation of PRSP2 and NAMPT would increase NADPH synthesis and availability for mitochondrial ATP production. A powerful stimulus for AMPK expression is an increase in the AMP to ATP ratio, indicating that ATP production is not keeping up with demand. Second, PRSP2, a critical enzyme phosphoribosyl pyrophosphate synthetase, is followed by NAMPT, that catalyzes the first and rate-limiting step in synthesis of purines, pyrimidines, tryptophan, and histidine. Purines and pyrimidines are well-known building blocks of DNA and RNA. The increase in NADPH availability would increase NADH-
linked substrate entry to the respiratory chain at complex I of the respiratory chain (Fig. 2B). The yield of ATP from NADH-linked and FADH$_2$-linked substrates is shown at the bottom of Fig. 2B. The asterisk next to complex I in the figure is a reminder that 7 of 41 genes responsible for synthesis of proteins in complex I were upregulated in the high-FE phenotype. Greater activity of all 5 complexes in the electron transport chain in the high-FE broiler phenotype (Bottje et al., 2006) and low-RFI sheep (Sharifabadi et al., 2012) has been reported. Although oxidative phosphorylation was not impaired in the low-FE phenotype (Bottje et al., 2002; Iqbal et al., 2004, 2005; Ojano-Dirain et al., 2004), this does not necessarily conflict with the discussion provided above. The ADP to oxygen ratio is typically assessed in isolated mitochondria removed from their cellular environment, constantly stirred, and with energy substrates provided in excess (Estabrook, 1967). Active respiration (state 3) is measured when ADP is in great excess and when all ADP has been converted to ATP (resting or state 4 respiration). In intact cells, the mitochondrial respiration probably exists between fully active state 3 respiration and fully resting state 4 respiration rate (Dranka et al., 2010). The point of this discussion is that although the ADP:oxygen ratio is a valuable index of mitochondrial function, it may not fully represent mitochondrial function that occurs in a cellular milieu.

Upregulated genes in the high-FE phenotype muscle are shown in a stylized cell in Fig. 3. As indicated above, AMPK and PRAKG2 are stimulated by energy demand (increased AMP:ATP) and increase expression of nuclear transcription factors PGC1-α and PPAR-γ. In turn, PGC1-α and PPAR-γ stimulate mitochondrial biogenesis, a process aimed to match cellular energy production with energy need triggered by AMPK. Also, AMPK stimulates 3 energy producing pathways: glycolysis, fatty acid oxidation, and oxidative phosphorylation. The glycolytic pathway would be favored in the high-FE phenotype because aldolase genes help anchor the glucose transporter 4 protein (GLUT4; Kao et al., 1999). Fatty acid oxidation would be enhanced in the high-FE phenotype by complement C1q tumor necrosis factor-related protein 3 (C1QTNF3) expression. Damaging effects from aldehydes and acetone (by-products of fat metabolism or lipid peroxidation) would be attenuated by glutathione S-transferase zeta 1 (GSTZ1) and maleylacetate isomerase (MAAI). Three upregulated genes in the high-FE phenotype, AMPK, PP2A, and APIP, would also decrease cell turnover by inhibiting apoptosis. Decreasing or delaying apoptosis would translate into increased energetic efficiency as well. Protein synthesis and protein activity in the high-FE phenotype would be enhanced by upregulation of GOLGB1, DCUN1 (see Table 1), and carbonic anhydrases 1 and 4.

The upregulation of anabolic-related genes contrasts with genes that were upregulated in the low-FE phenotype (Fig. 4). The gene exhibiting the greatest differential expression was CSRP3, which is found in both the nucleus and cytosol (Flick and Konieczny, 2000). Because CSRP3 stimulates myogenic differentiation, upregulation of many cytoarchitecture and muscle fiber genes in the low-FE phenotype may have been in response to CSRP3.

Mitochondrial ROS production from site-specific defects in electron transport is prevalent in mitochondria obtained from the low-FE phenotype (Bottje et al., 2002; Ojano-Dirain et al., 2004; Iqbal et al., 2005). Increased HSP90, Hsp27, and HSPB7 expression may have been due to increased mitochondrial ROS production. As mitochondrial ROS also induces SOD (Yu, 1994), increased Mn-SOD (mitochondrial) and Cu-Zn SOD (cytosolic) expression may also be attributed to increased mitochondrial ROS production.

Suppressor of the cytokine signaling 3 (SOCS3) was also upregulated in the low-FE phenotype. The SOCS family encoding proteins exert feedback inhibi-
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bition of signaling pathways induced by hormones, growth factors, and cytokines (Fujimoto and Naka, 2003). Signaling pathways modulated by SOCS3 are tissue specific and function 1) to modulate feed intake by inhibiting leptin signaling (Bjørbaek et al., 2000; Dunn et al., 2005), 2) to modulate GH and cytokine pathways (Lindberg et al., 2005; Rønn et al., 2008), and 3) to attenuate insulin activity by insulin receptor phosphorylation (Emanuelli et al., 2000; Ueki et al., 2004). Increased SOCS3 expression in type II muscle fibers caused dilation of the sarcoplasmic reticulum and swollen mitochondria (Lebrun et al., 2009). As shown in Fig. 4, increased SOCS3 expression could attenuate PDGF signaling in the low-FE phenotype and could be deleterious to mitochondrial structure integrity. In adipocytes, increased SOCS3 expression decreases insulin signaling without affecting systemic insulin resistance (Shi et al., 2006). Constitutive SOCS3 expression in transgenic mice established a potential role of SOCS3 in muscle structure and locomotor activity (Lebrun et al., 2009). Thus, increased expression of SOCS3 may modulate mitochondrial function and/or growth factors and cytokine signaling in the low-FE phenotype.

Additional groups of genes that were upregulated in the low-FE phenotype included PDGF, GPCR, and major histocompatibility complex II. Although three GPCR (P2RY13, RGR, CSR) genes were increased in the low-FE phenotype, their effect on cell function is unclear because several downstream genes in the Jnk or PKA pathways were downregulated in the low-FE phenotype. Additional groups of genes that were upregulated in the high-FE phenotype included cellular bioenergetics, protein synthesis, packaging, and structure and in detoxification of metabolic by-products. Genes that were upregulated in the high-FE phenotype are shown in open circles (in red). An increase in the AMP to ATP ratio would be sensed by adenosine monophosphate protein kinase (AMPK) activity that increases energy production in the cell by activating PPAR-γ and PPAR-γ coactivator-1 α (PGC1-α), which increase mitochondrial numbers in the cell (mitochondria biogenesis). The increase in AMPK activity would also increase energy output in the cell by stimulating glycolysis, oxidative phosphorylation, and fatty acid oxidation. Aldolase (Aldo) helps anchor glucose 4 transporting protein (GLUT4) to the cell membrane, which would enhance glucose entry into the muscle cell. Potentially damaging by-products of metabolism from aldehydes, ketones, and reactive oxygen species (ROS) would be metabolized by aldehyde dehydrogenase 1 family member A (ALDH1A), glutathione S-transferase zeta 1 (GSTZ1), and maleylacetoacetate isomerase isomerase (MAAI) as well as glutathione peroxidase (GPx). The protein kinase A (Pka) cascade along with Rat sarcoma proteins (Ras) and retinoid X receptor (RAR-RXR) pathways would increase Jnk activity, which would enhance transcription. In the nucleus, stress-activated protein kinase (Sapk) is similar to Jnk. Increased transcription would in turn increase translation of cellular proteins. Protein packaging would be enhanced by the activity of GOLGB1. Activity of translated proteins in the cell would be enhanced by scaffolding function provided by activities of Aldolase, DCUN1 (Table 1), and carbonic anhydrase (CA) 1 and CA4. See online version to view figure in color.

Figure 3. Processes potentially enhanced in breast muscle of the high feed efficiency (FE) broiler phenotype include cellular bioenergetics, protein synthesis, packaging, and structure and in detoxification of metabolic by-products. Genes that were upregulated in the high-FE phenotype are shown in open circles (in red). An increase in the AMP to ATP ratio would be sensed by adenosine monophosphate protein kinase (AMPK) activity that increases energy production in the cell by activating PPAR-γ and PPAR-γ coactivator-1 α (PGC1-α), which increase mitochondrial numbers in the cell (mitochondria biogenesis). The increase in AMPK activity would also increase energy output in the cell by stimulating glycolysis, oxidative phosphorylation, and fatty acid oxidation. Aldolase (Aldo) helps anchor glucose 4 transporting protein (GLUT4) to the cell membrane, which would enhance glucose entry into the muscle cell. Potentially damaging by-products of metabolism from aldehydes, ketones, and reactive oxygen species (ROS) would be metabolized by aldehyde dehydrogenase 1 family member A (ALDH1A), glutathione S-transferase zeta 1 (GSTZ1), and maleylacetoacetate isomerase isomerase (MAAI) as well as glutathione peroxidase (GPx). The protein kinase A (Pka) cascade along with Rat sarcoma proteins (Ras) and retinoid X receptor (RAR-RXR) pathways would increase Jnk activity, which would enhance transcription. In the nucleus, stress-activated protein kinase (Sapk) is similar to Jnk. Increased transcription would in turn increase translation of cellular proteins. Protein packaging would be enhanced by the activity of GOLGB1. Activity of translated proteins in the cell would be enhanced by scaffolding function provided by activities of Aldolase, DCUN1 (Table 1), and carbonic anhydrase (CA) 1 and CA4. See online version to view figure in color.
we feel that these studies are important in adding knowledge that may ultimately enhance animal production to help feed a hungry world.

Increased PDGF expression might enhance muscle growth and development and may facilitate increased expression of myofibers or cytoskeletal muscle fibers along with CSRP3. Additional evidence of potential cellular stress in the low-FE phenotype is provided by the upregulation of SGK1 and CRH.

We hope this review provides a potential road map to aid genetic selection of food animals and add a few more pieces to the FE puzzle. We are continuing to examine and analyze the global gene expression related to FE. Although the data are limited to muscle tissue and do not address aspects of FE at the whole animal level, we feel that these studies are important in adding knowledge that may ultimately enhance animal production to help feed a hungry world.


Hopkins, S., L. Lackner, and J. Nunnari. 2007. The machines that di...


