

# Environmental drivers of mitochondrial content and its role in feed efficiency and meat quality

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2017 Kemin Total Nutrition Conference

Session: Environmental factors can not be ignored in animal production

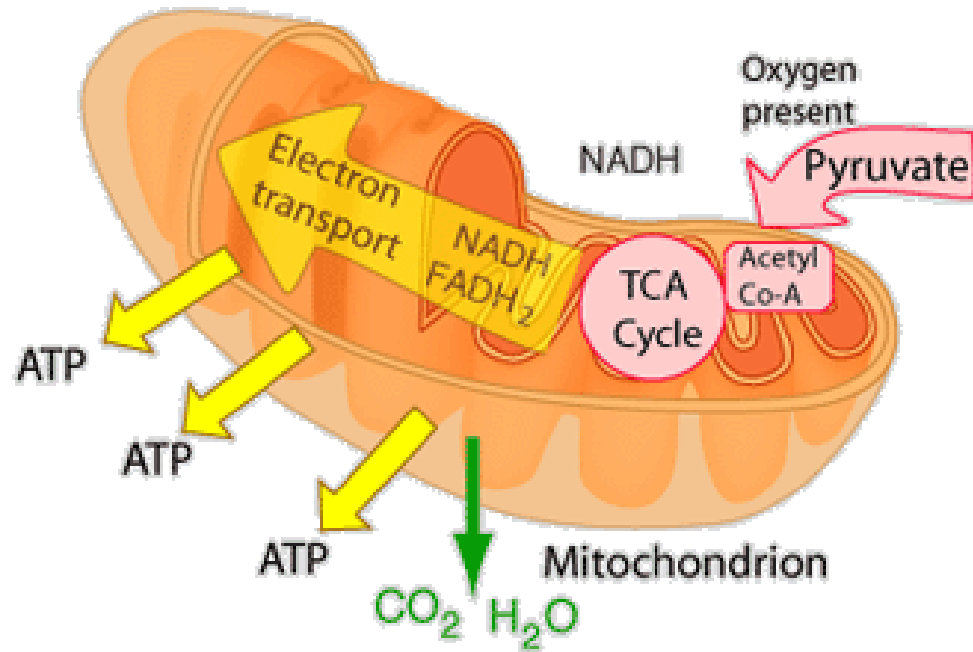
# Overview

- Why tissue mitochondrial content?
  - Feed efficiency (low mitochondrial content, muscularity and high FE)
  - Meat quality (mitochondria and post-mortem metabolism?)
- Genetic and **environmental** drivers of high mitochondrial content
  - Cold - ambient temperature (thermogenic tissues muscle and brown fat)
  - Diet - high fat, ketone esters, curcumin, PQQ
  - Activity - endurance exercise
- Illustrate with multiple production species, special focus on broilers

# Aim

- Start a discussion about mitochondrial phenotypes in animal production and how they may be environmentally influenced in commercial settings:
  - Previous work with Cobb Vantress (broiler mitochondrial content variation)?
  - Previous work with USDA and University of Arkansas (molecular mechanisms of divergence in broiler FE, role for the mitochondrion)
  - New project proposal with Kemin and CAS (pig heat stress and molecular / mitochondrial mechanisms through which nutrition may provide mitigation)

# Mitochondria and energy flow

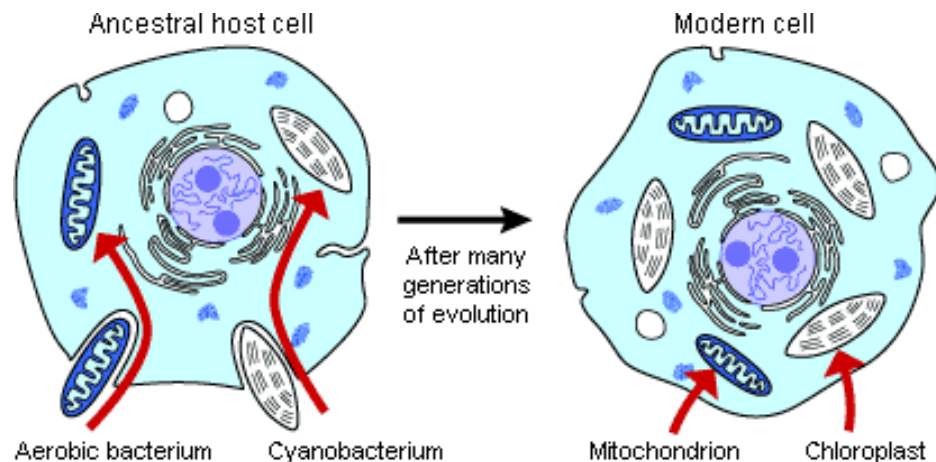


A mitochondria is like a bicycle, converting one source of energy into another through a coupling mechanism.

Both the size of the bicycle and the coupling mechanism can be adapted to meet different metabolic goals.

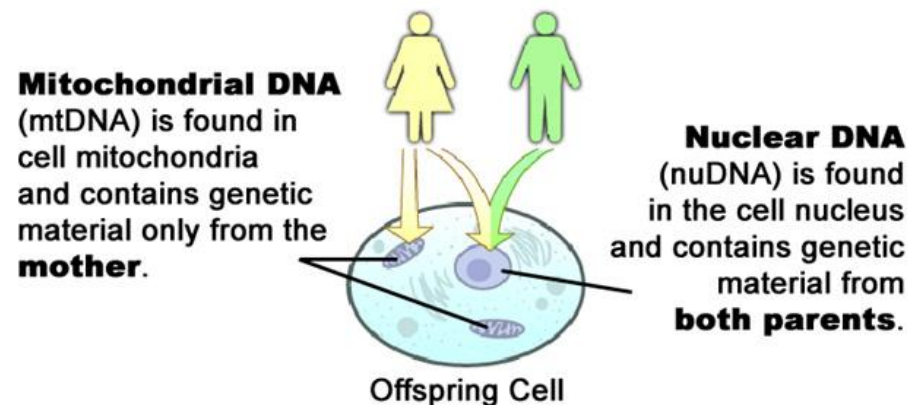
# What is a mitochondria?

- Complex, cellular organelle comprising ~1000 proteins
- The 'engine' of the cell
- Thought to be formerly a free-living bacteria that has formed a symbiotic relationship with the host cell of modern animals.

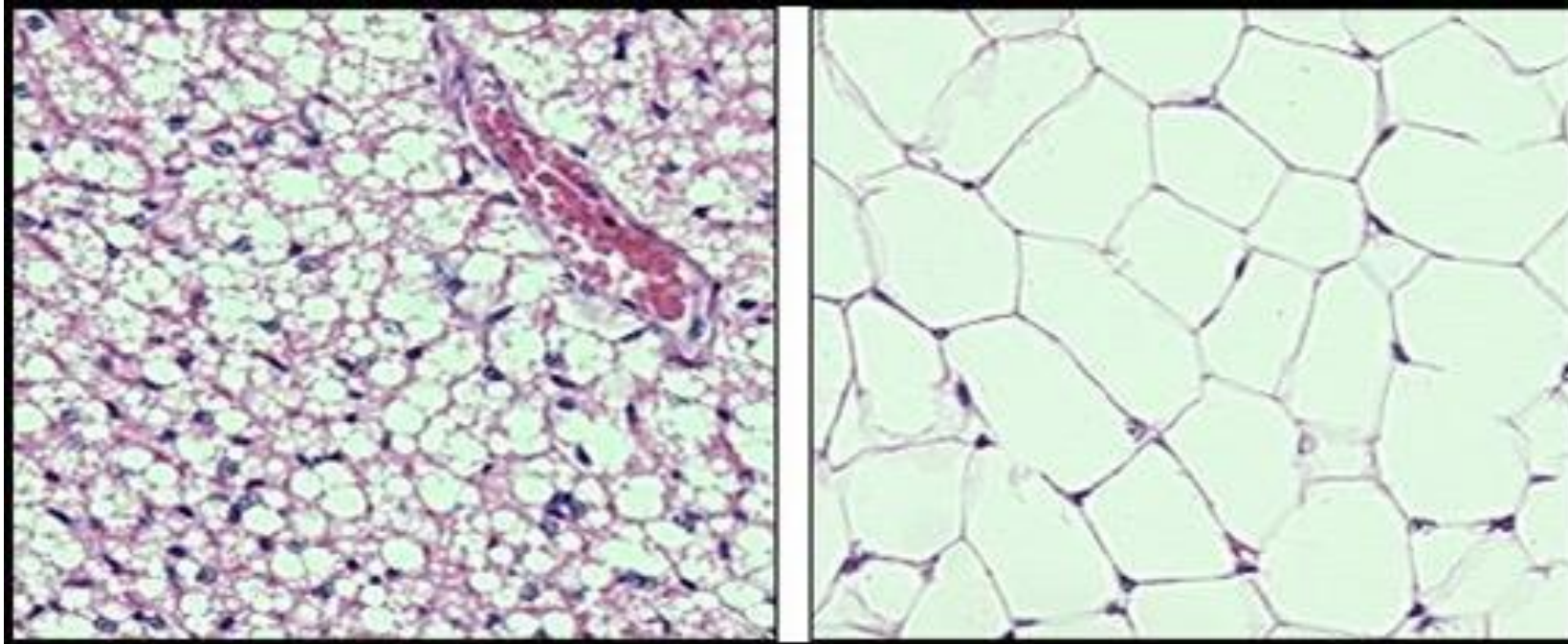


# An animal cell: two genomes in cooperation

- Mitochondria retains some of its own genome, encoding 13 protein coding genes plus other machinery (DNA based molecular biotech can be used).
- Remaining (majority) mRNA encoding mitochondrial proteins are in the nuclear genome.
- The collective expression of the mRNA that encode the mitochondria is controlled by a combination of both environment and genetics



# Mitochondria in action – brown versus white fat



Brown fat important for cold stressed neonatal mammals and has a likely role in feed efficiency

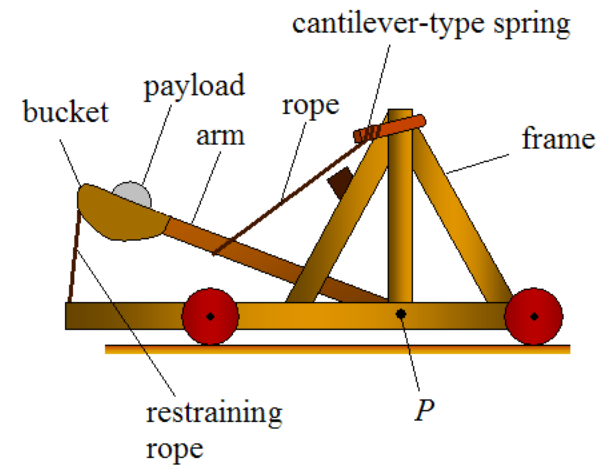
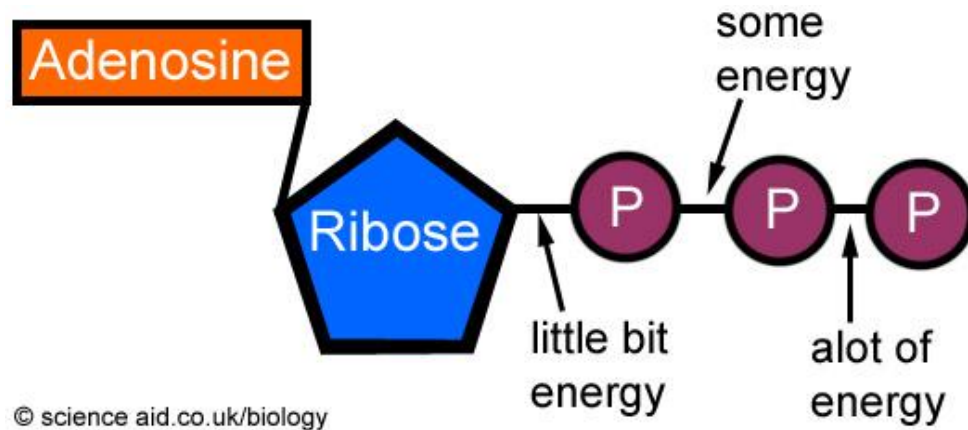
# Why think about mitochondria?

- The 'engine' of the cell where the majority of feed energy is converted into biologically usable ATP (and some body heat).
- ATP ultimately pays for production, such as deposition of lean.
- Improvements in mitochondrial performance (food → ATP conversion) will improve whole animal feed efficiency.
- Improvements can be made by both environmental and genetic mechanisms.

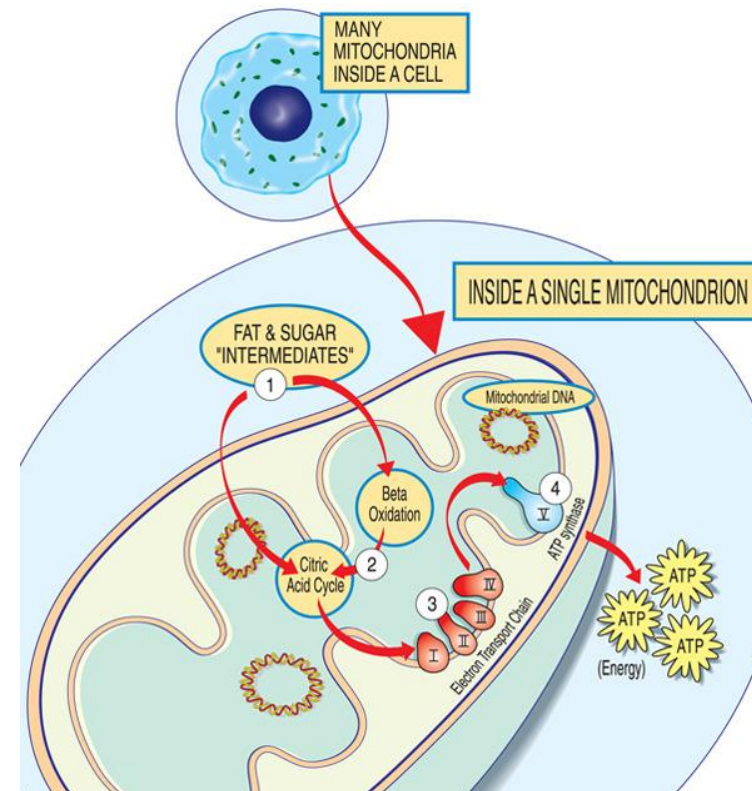
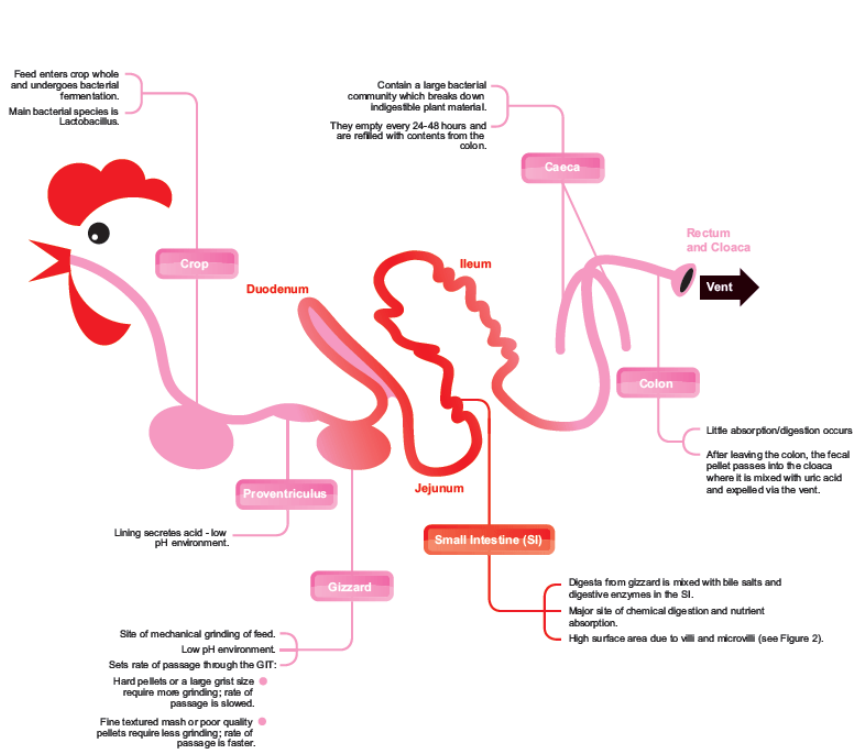


# ATP – the 5 cent coin of bioenergetics

- Dietary glucose, protein and fat can be seen \$100 notes
- If a biological process costs 10 cents, more efficient to pay with two 5 cent coins than breaking a big note and ‘losing the change.’



# Feed, digestion and energy conversion in the mitochondria



Where is the useful variation across populations of animals? Gut or **muscle**?

# Mitochondrial adaptations, energy and heat

Fuel  
Sources

Glucose  
Fatty Acids  
Amino Acids

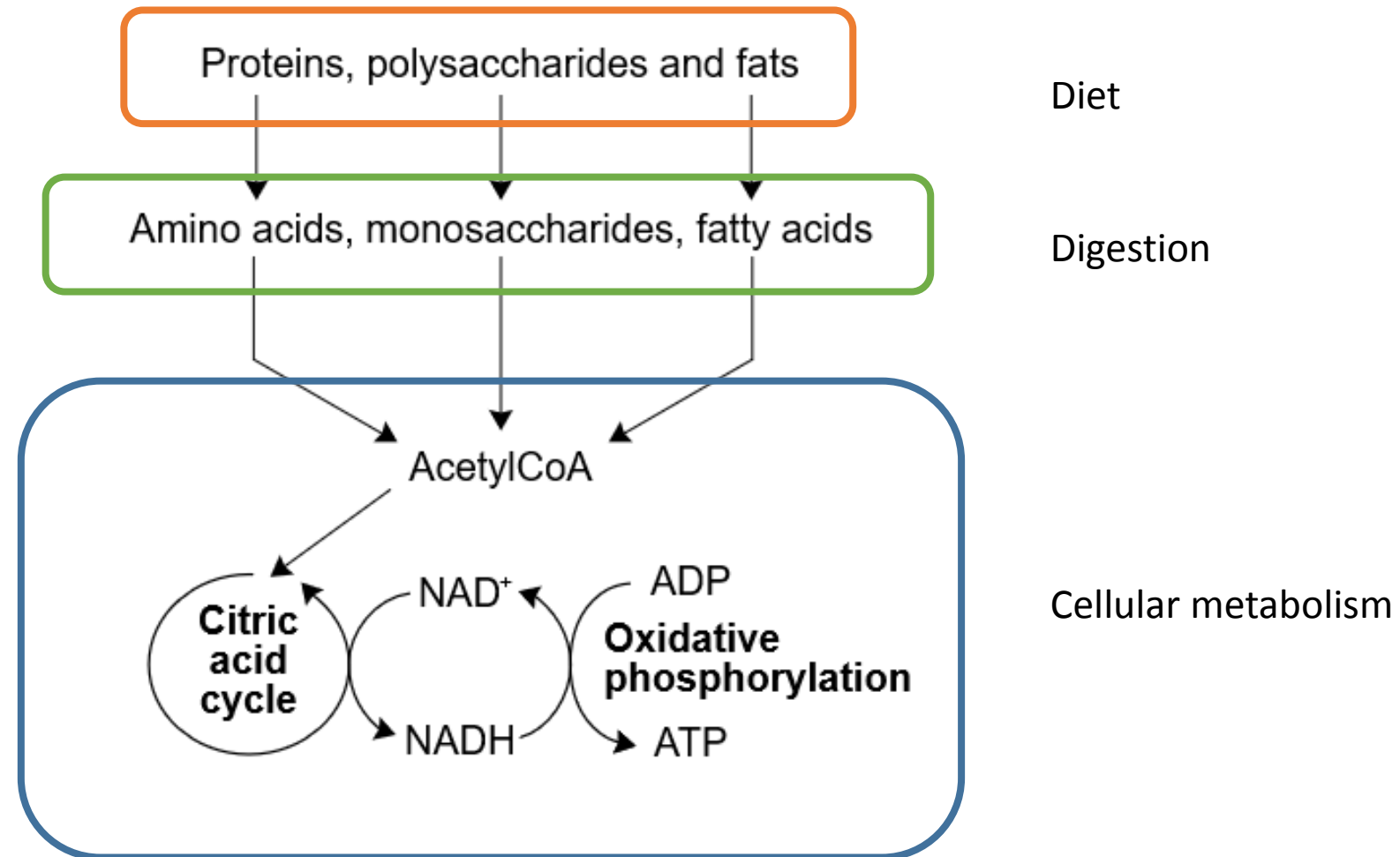


➔ ATP



- Brown fat has a large bicycle (high energy flux) with no chain (no ATP, lots of heat).
- White fat has a small bicycle (low energy flux) with a chain (little heat, little ATP).
- Heart muscle has a large bicycle (high energy flux) with a chain (lots of ATP)

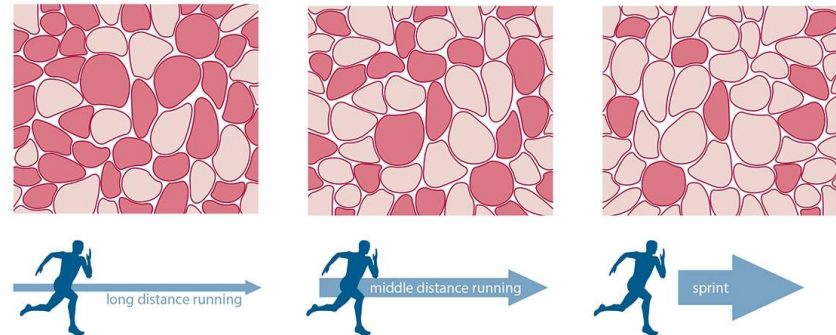
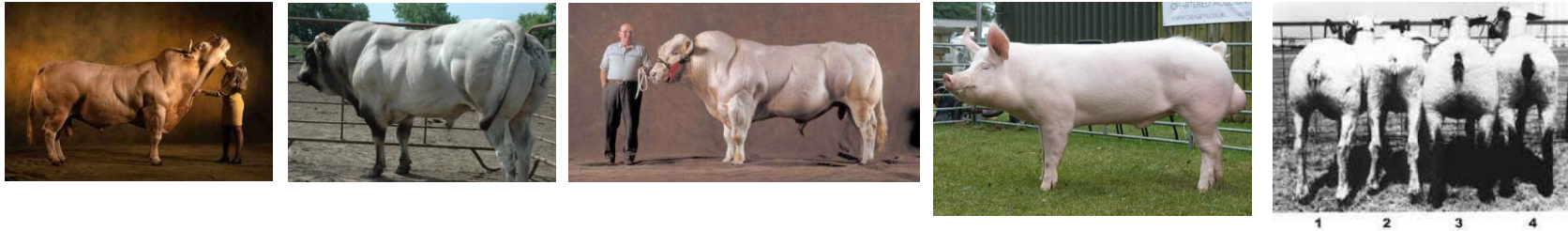
# How do mitochondria convert energy?



# Muscle mitochondrial content

- If the mitochondria is the engine of the cell, then mitochondrial content is engine size.
- Focus on muscle because it is 50% of the animals mass, a substantial contributor to whole animal metabolism, is composed of fibres with different mitochondrial contents and is the tissue we consume in animal production systems
- How might muscle mitochondrial content (engine size) influence production?
- What environmental factors drive mitochondrial content (engine size)

# Feed efficient breeds tend to be built of low mitochondrial content type IIB muscle



# 'Corolla' chicken versus 'drag racing' hummingbird

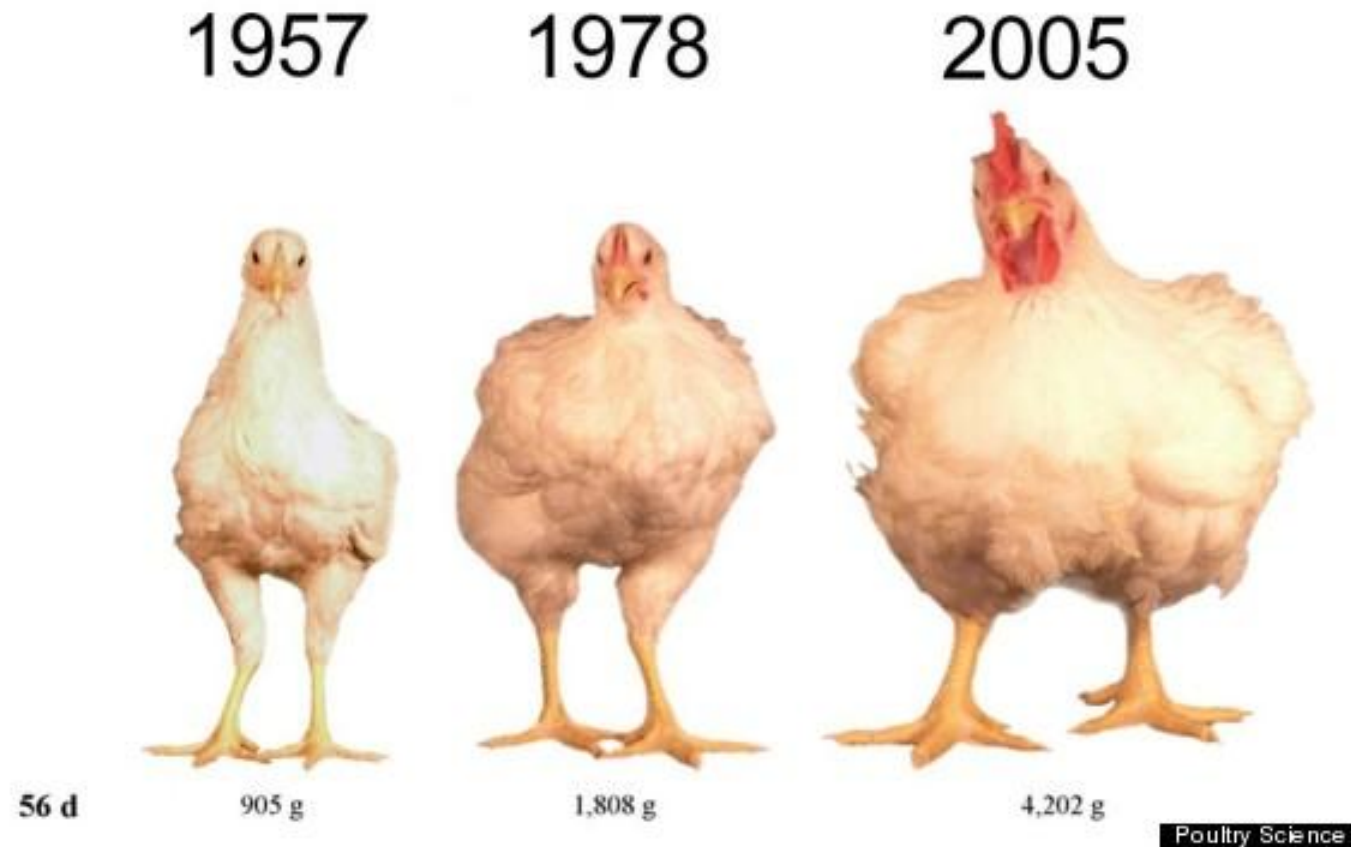


Exceptional feed conversion  
Sedentary  
*Pectoralis* mitochondrial content **4%**  
Small engine 'Toyota Corolla'



Hibernation  
Athletic  
35%  
'Gas guzzling' drag racer

# The transformed broiler – a ~50 year story

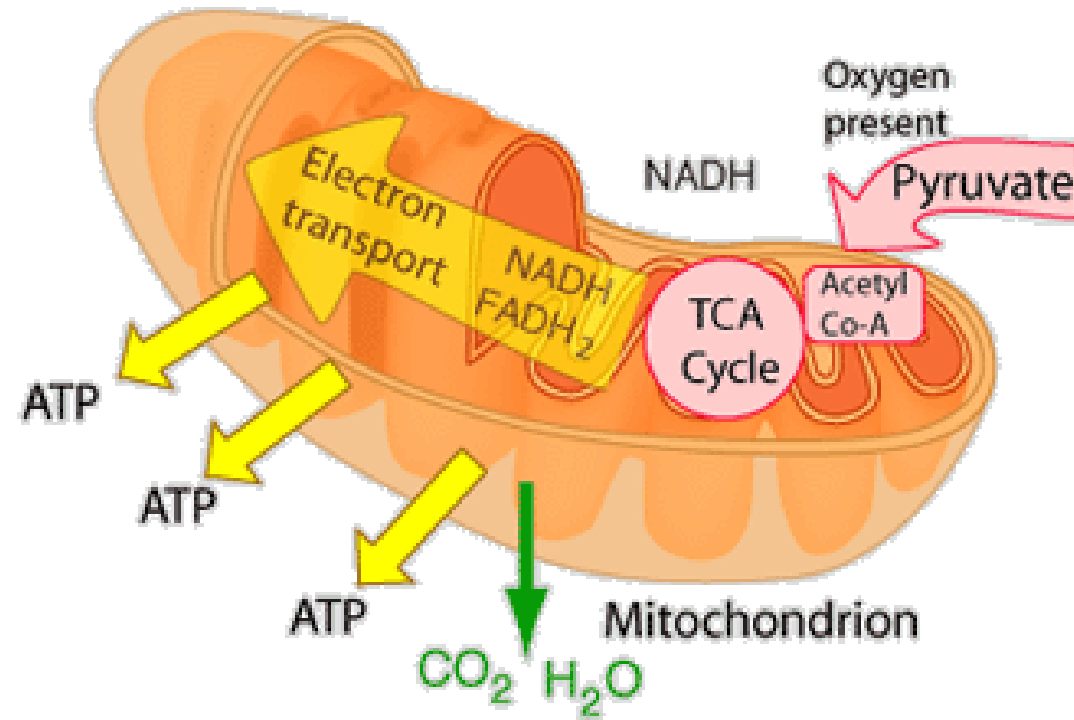




# Genetics and environment in broiler production

- **Genetic** gain: breeding from genetically superior parents, now using DNA markers to inform the selection process
- **Environmental** change: nutrition and other management practices
- Collectively, improve the **efficiency of the flow of energy** into lean tissue deposition, primarily the enlarged breast muscle

# Efficiency, flow, energy



# Overview of recent broiler research

- Measuring broiler mitochondrial phenotypes (using DNA and RNA)
- Industrial (Cobb Vantress, HQ Arkansas, USA) and academic colleagues (University of Arkansas, USA)
- Both projects used Cobb Vantress broilers as the animal resource.

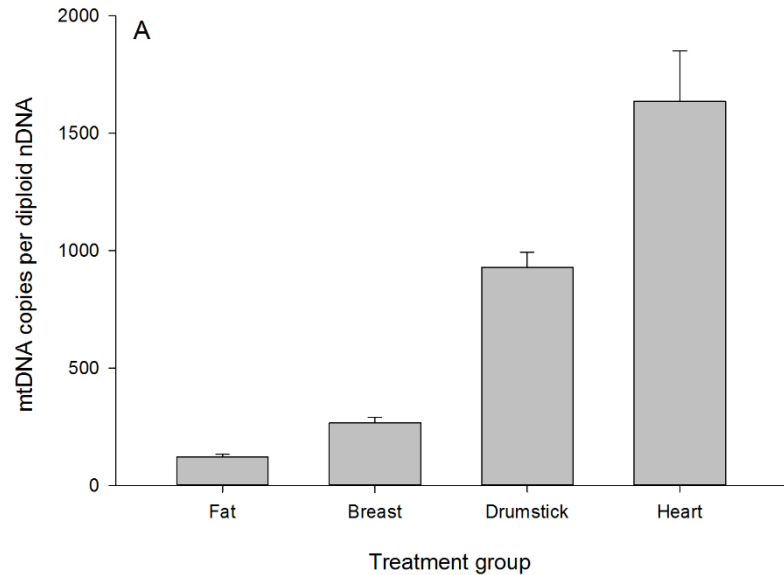
# Cobb Vantress

- US multi-national company that produces broilers for people who produce broilers.
- Control the genetics for ~50% of the global supply of broilers.
- Genetic improvement with a particular emphasis on feed efficiency
- My role was to measure a cellular phenotype (mitochondrial content) that is the product of bird environment as well as bird genetics

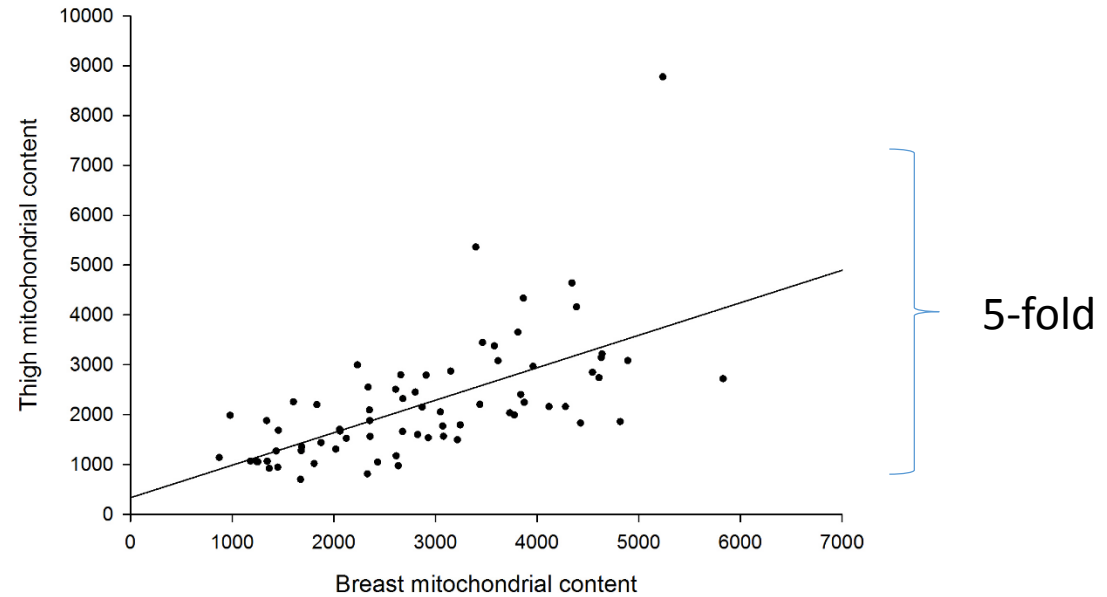
# Broiler mitochondrial content

- Screen birds (within a genetic line) for variation in muscle mitochondrial content. How much spread around the 4%?
- Develop the biotechnology to achieve this (qPCR from DNA).
- Connect any variation to 11 performance phenotypes (but not FE).

# Broiler mitochondrial content



2%      4%      12%      24%



The assay can discriminate tissues with different metabolic demands.

There is a positive relationship ( $0.61$ ;  $P < 0.0001$ ) between breast and thigh content across 80 birds.

# Mitochondrial content and performance traits

- Breast muscle mitochondrial content negatively correlated with:
  - Breast muscle yield (-0.27;  $P = 0.037$ )
  - Carcass yield (-0.26;  $P = 0.045$ )
  - Abdominal fat content (-0.31;  $P = 0.017$ )
- Thigh muscle mitochondrial content negatively correlated with:
  - Bow out leg defect (-0.30;  $P = 0.011$ )

# Regulation of muscle mitochondrial content

- **Genetics**

- species comparisons, domestication and industrial selection

- **Environment**

- Exercise (endurance versus sprint exercise)
- Ambient temperature (heat versus cold)
- Diet (high fat, ketone esters, curcumin and PQQ)

- **G and E integrated** by a transcriptional regulator called *PPARGC1A*



# Summary of Cobb Vantress research

- There appears to be systemic regulation of muscle mitochondrial content in chickens. A bird with a low value for thigh, will also have a low value for breast.
- A bird with low breast mitochondrial content tends to be more muscular with higher abdominal fat.
- A bird with low thigh mitochondrial content tends to be more likely to express the bow out leg pathology.

# Mitochondrial content in broilers

© 2017. Published by The Company of Biologists Ltd | Biology Open (2017) 6, 50-58 doi:10.1242/bio.022772



## RESEARCH ARTICLE

### Chicken muscle mitochondrial content appears co-ordinately regulated and is associated with performance phenotypes

Antonio Reverter<sup>1</sup>, Ron Okimoto<sup>2</sup>, Robyn Sapp<sup>2</sup>, Walter G. Bottje<sup>3</sup>, Rachel Hawken<sup>2</sup> and Nicholas J. Hudson<sup>4,\*</sup>

#### ABSTRACT

Mitochondrial content is a fundamental cellular bioenergetic phenotype. Previous work has hypothesised possible links between variation in muscle mitochondrial content and animal performance. However, no population screens have been performed in any production species. Here, we have designed a high throughput molecular approach to estimate mitochondrial content in commercial broilers. Technical validity was established using several approaches, including its performance in monoclonal DF-1 cells, cross-tissue comparisons in tissues with differing metabolic demands (white fat < breast muscle < drumstick muscle < heart muscle) and, as a negative control, a near absence of mtDNA amplification from whole blood. We screened breast muscle and thigh muscle in 80 birds individually phenotyped for 11 growth and development traits. Substantial individual variation (fivefold) was discovered in both breast and thigh muscle mitochondrial content. Interestingly, across birds we detected a very strong positive relationship between breast and thigh content (correlation coefficient 0.61;  $P < 0.0001$ ), consistent with coordinate regulatory control across the musculature. Further, breast muscle mitochondrial content is negatively correlated with breast muscle yield ( $-0.27$ ;  $P = 0.037$ ), abdominal fat content ( $-0.31$ ;  $P = 0.017$ ) and carcass yield ( $-0.26$ ;  $P = 0.045$ ). Therefore, low breast muscle mitochondrial content is associated with more muscular birds possessing higher abdominal fat, the latter being in line with biomedical models of obesity. Finally, thigh mitochondrial content is

phenotypes. In the post-genomics era it has exploited single nucleotide polymorphism (SNP) genotyping to enable DNA marker-assisted selection practices (Lahav et al., 2006). The combination of the two practices has transformed domestic chickens over the last century to the modern animals with their precocious growth rates, dramatically increased muscle mass and markedly improved feed efficiencies (Paxton et al., 2010; Siegel, 2014).

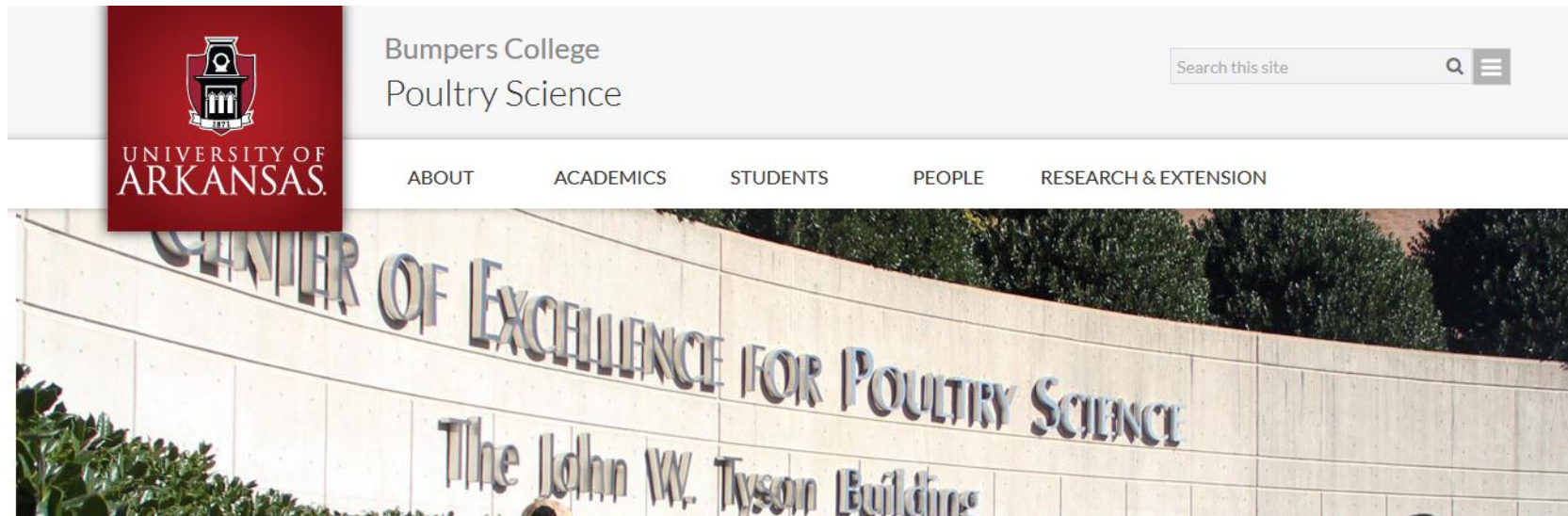
In principle, the DNA-based selection approach can mitigate the need to measure expensive phenotypes such as feed efficiency, or make predictions about individuals that do not directly express the phenotype, such as the contribution of male genetics to female reproductive characteristics. However, predicting phenotype from genotype is challenging even for industries that have an animal resource with a small effective population size and high levels of inbreeding. In chickens, the current accuracy of genomic prediction for a typical complex trait of moderate heritability is 54% (Lourenco et al., 2015). This is strong enough for implementation in a breeding strategy but there would be value in further improvements. One possible avenue is to develop biomarkers complementary to DNA sequence information. If these are practical and economical, they could be implemented in parallel to genetic testing and the two sources of information integrated for stronger predictions. Alternatively, any SNP subsequently found to be associated to the new biomarker could help refine the existing genomic predictions and therefore be delivered through the current DNA prediction

# Q-CAS grant submitted February 2017

- Queensland (Uni Qld colleagues) and Chinese Academy of Science (Dr XiangFeng Kong) collaboration
  - Kemin China (Dr. Bing Guo) are the industrial co-funder
  - Explore the molecular basis of nutritional treatments (betaine and curcumin / essential plant oils) that may mitigate heat stress and improve production under sub-tropical conditions
- One part of the project is to develop a mitochondrial content assay for pigs, analogous to the industrial broiler research just described

# University of Arkansas

- Prof. Walter Bottje, Centre of Excellence for Poultry Science



# University of Arkansas and genome-wide gene expression

- Develop a deeper understanding of feed efficiency and its relationship to muscle structure and mitochondrial metabolism (Cobb broilers)
- Use genome-wide gene expression as the tool
- Observed patterns of gene expression are a complex consequence of environment and genetics

# University of Arkansas and genome-wide gene expression

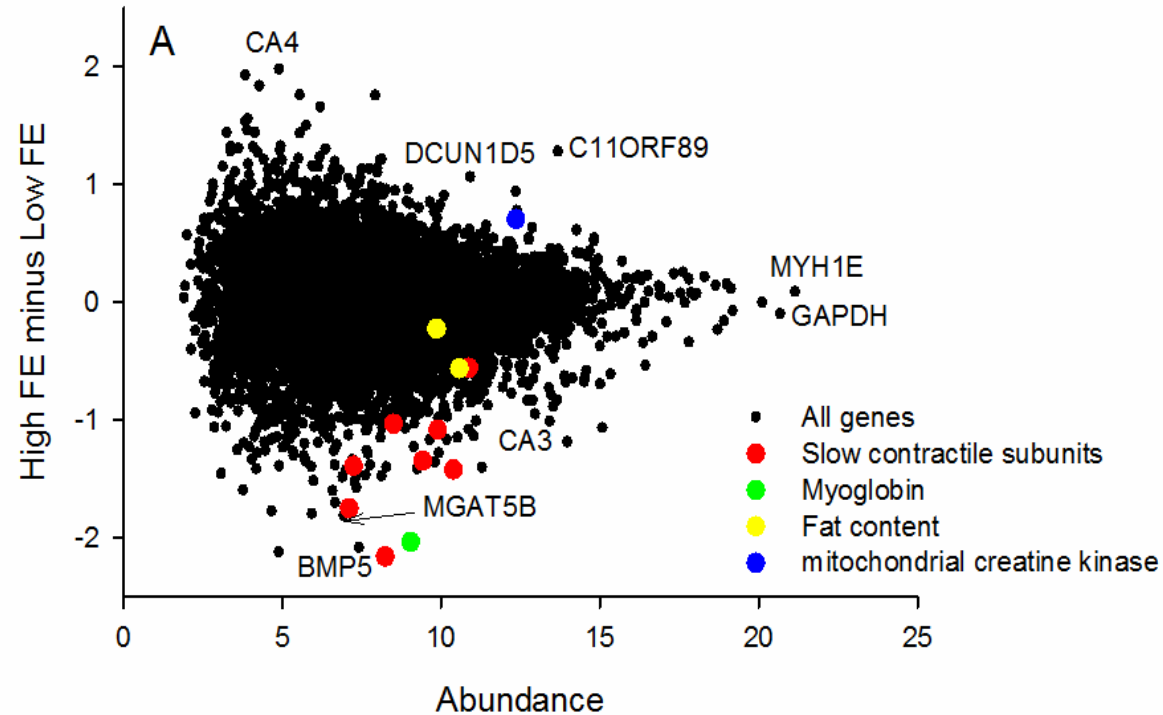
- Within a single genetic line, birds phenotyped for FE between 6 and 7 weeks, (1.5 fold difference).
- Breast muscle RNA extracted (n = 6 in each of two groups).
- Genome-wide mRNA quantified. **This gives a global picture of ~10,000 gene expression values.**
- Some of these genes encode proteins relating to muscle structure, mitochondrial function and metabolism.

# Drowning in data

14502	A_73_120244	FBXO11	Y	12.3025	12.8356	12.4357	12.1377	12.3776	12.7111	12.4237	12.3494	12.3051	12.7244	12.4922	12.4
14503	A_73_120245	LTB4R2	Y	6.06182	5.75777	5.99576	6.22621	5.65644	5.76563	5.68533	5.4969	5.97771	6.05889	6.14086	5.8
14504	A_73_120246	MRPS24	Y	12.8914	13.2978	13.181	11.532	12.8201	12.723	13.3142	12.6336	13.0752	12.8598	13.3097	13.1
14505	A_73_120247	RASAL2	Y	6.40008	6.04797	4.70939	5.5717	4.84951	5.08052	4.61057	4.3082	4.51624	4.84972	6.1868	5.5
14506	A_73_120249	RAB7A	Y	15.3736	16.1201	15.7261	15.3175	15.8505	16.1888	16.0098	15.6737	15.6727	16.1922	15.7201	15.6
14507	A_73_120250	EIF2AK3	Y	6.69056	6.41431	6.03634	4.79695	5.63787	5.32809	4.70103	4.69475	4.91894	4.86378	7.03819	6.4
14508	A_73_120251	NEDD4L	Y	10.6408	8.82865	8.82306	9.3776	8.32224	9.06235	9.66553	9.11071	9.09552	9.59845	10.1862	9.1
14509	A_73_120253	A_73_120253	Y	7.0448	6.98473	6.81664	6.00237	6.33256	6.82234	5.8353	5.64351	5.93596	6.6225	7.17678	7.1
14510	A_73_120254	MSTN	Y	8.72581	7.65877	8.63289	4.93011	7.79136	8.93952	8.98846	6.96958	7.77954	7.00617	9.34228	8.6
14511	A_73_120255	C14H8ORF70	Y	7.50045	6.87585	7.0741	3.64667	4.34447	4.72573	4.7688	4.15779	4.47055	4.46076	6.97353	7.1
14512	A_73_120257	GIMAP7	Y	2.53541	5.41354	4.88169	4.49988	6.98193	7.31226	7.57265	7.05273	7.49784	5.99213	2.99342	3.8
14513	A_73_120258	ALDH7A1	Y	10.4526	9.71446	8.7367	7.75682	8.95604	9.97182	9.57148	9.4855	9.38248	8.95401	9.97283	9.2
14514	A_73_120259	ADAMTS10	Y	6.7161	6.10551	6.40848	4.97768	4.65453	4.31532	3.57451	3.92325	4.31422	5.3358	6.48577	6.2
14515	A_73_120261	HSD17B7	Y	12.0477	11.6968	10.9042	10.0601	9.76593	9.7111	10.2038	9.87441	10.2706	10.4067	11.8753	11
14516	A_73_120262	MKI67IP	Y	10.4829	10.5049	9.43027	11.204	9.22997	9.02327	9.68939	8.7211	9.34814	9.54323	10.875	10.1
14517	A_73_120263	A_73_120263	Y	9.45541	7.86952	7.24895	6.18001	7.27421	6.98965	7.59376	7.37168	7.48281	7.82077	6.68413	5.5
14518	A_73_120264	KLHL21	Y	5.49553	5.61282	4.67782	4.36253	3.85963	3.38521	3.3125	2.94191	3.69872	3.72848	5.88035	5.2

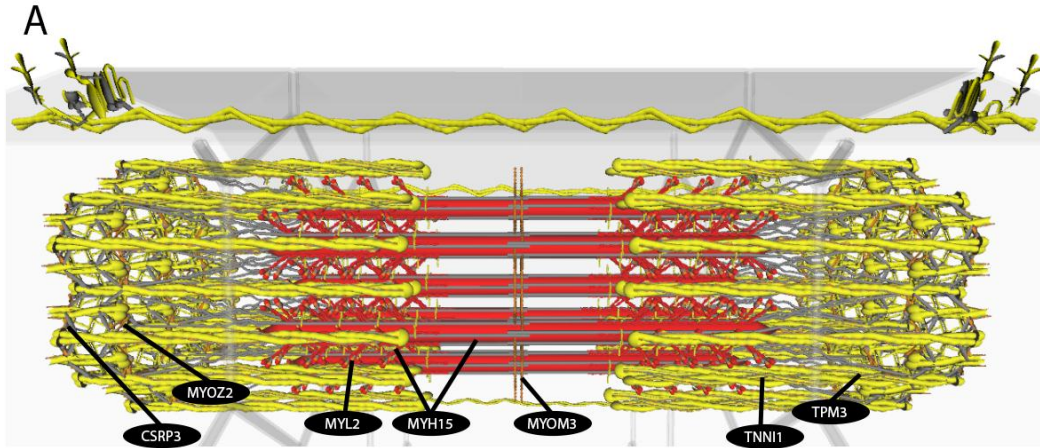


# University of Arkansas and genome-wide gene expression I



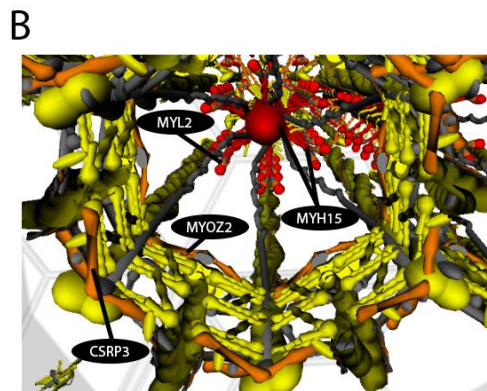


# University of Arkansas and genome-wide gene expression II



A 3d muscle model can be used to assess the physical structure of muscle

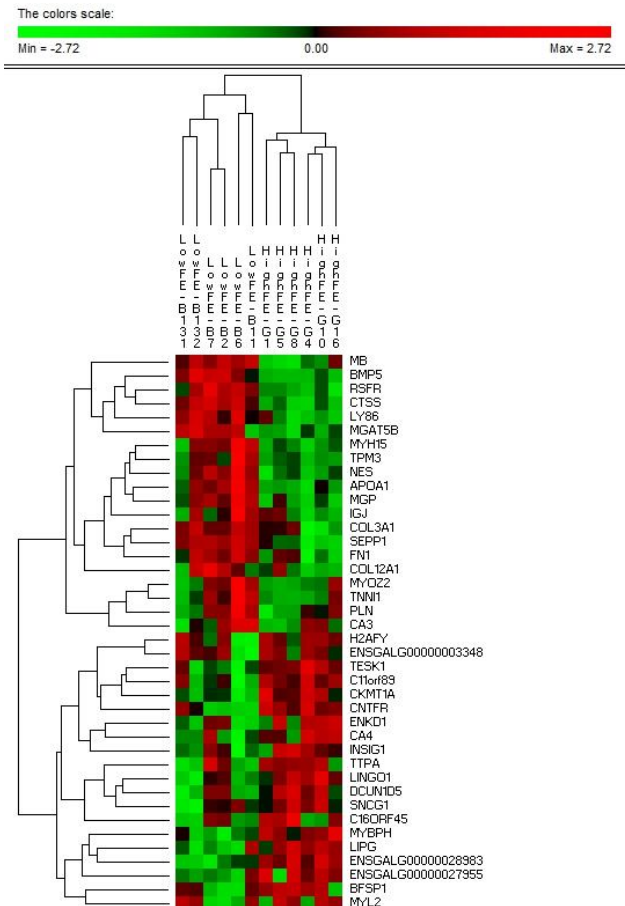
Fibre composition has changed, less slow, red fibres in the more efficient birds



C *Proteins highlighted in VMus3D*

Location	Gene ID	High FE - LowFE
thick filament	MYH15	-2.16
	MYL2	-1.08
	MYOM3	-1.03
thin filament	TNNI1	-1.75
	TPM3	-1.42
z-disk	MYOZ2	-1.34
	CSRP3	-1.39

# University of Arkansas and genome-wide gene expression III



Panel of 40 genes expressed in breast muscle can discriminate the 12 birds into treatment group i.e. high versus low efficient

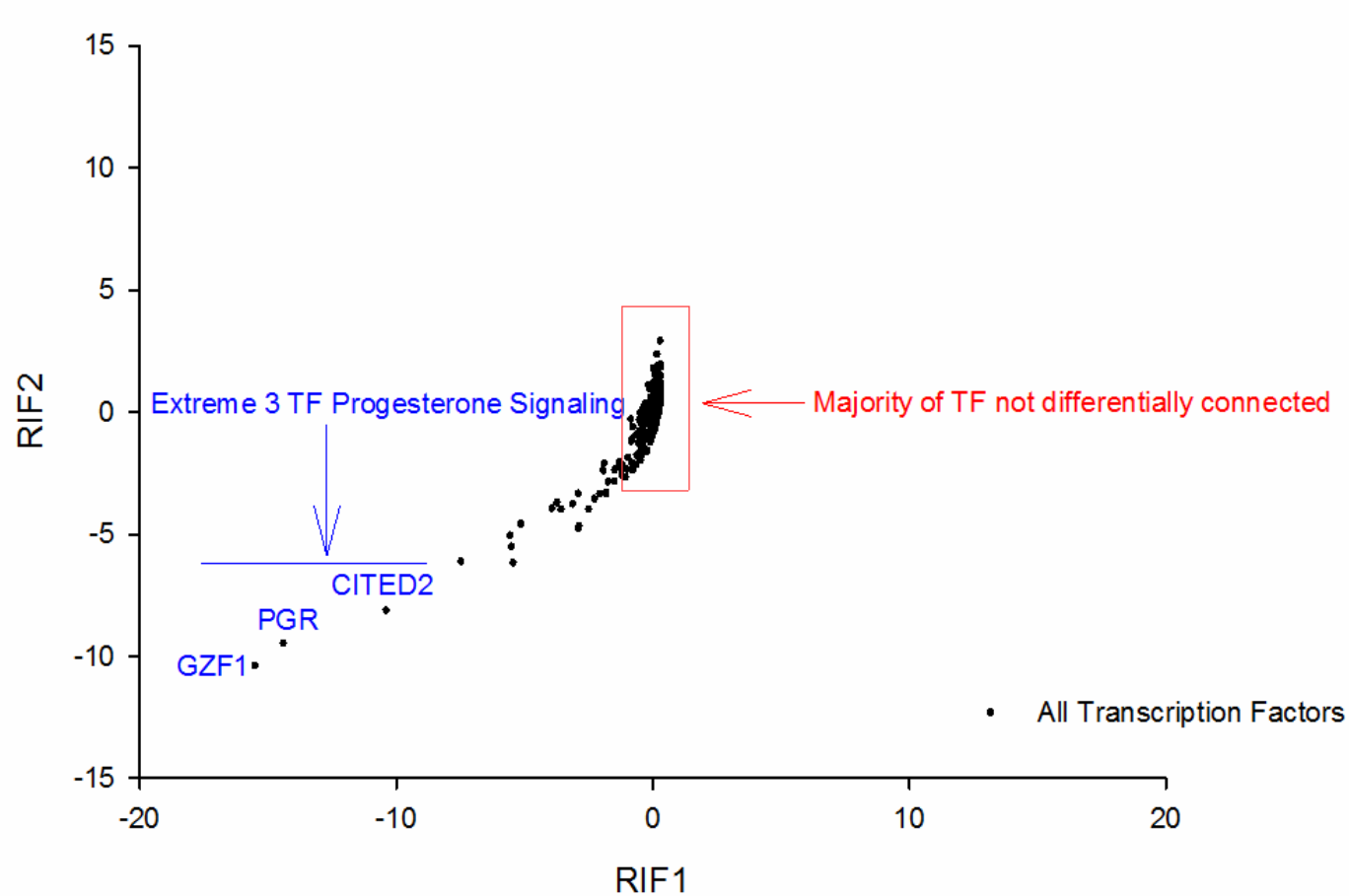
More efficient birds express less slow, red muscle (e.g. MYL2, MB, TNNT1, MYOZ2, TPM3, MYH15) and have altered mitochondrial metabolism (e.g. CKMT1A).

# Differential network analysis



Hudson et al (2009). *PLoS Computational Biology*. 5(5):e100382

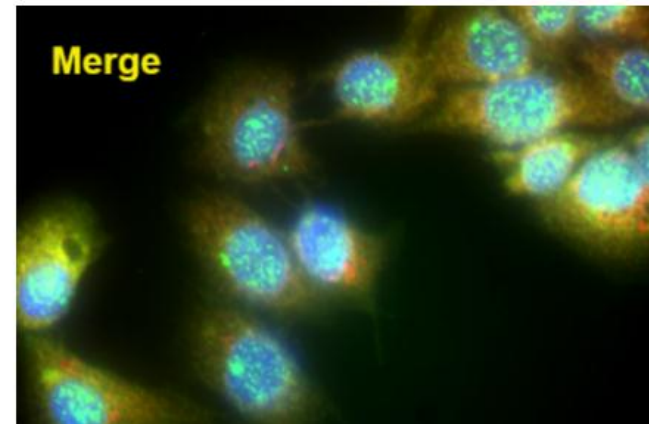
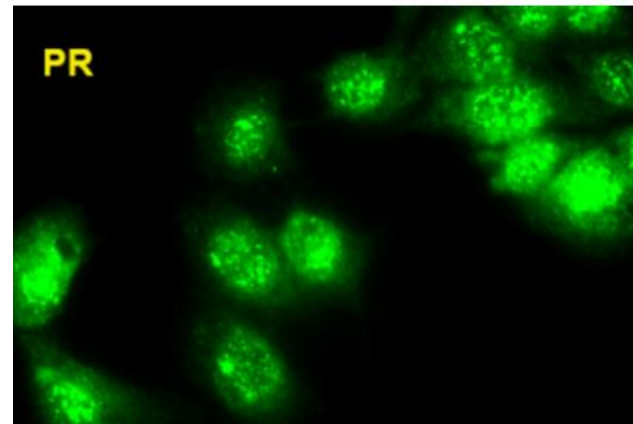
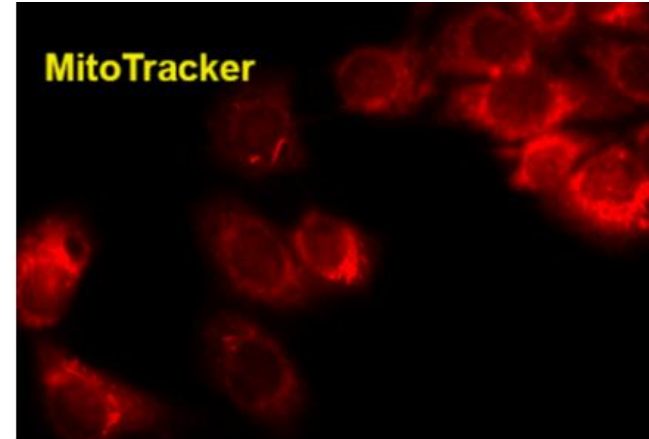
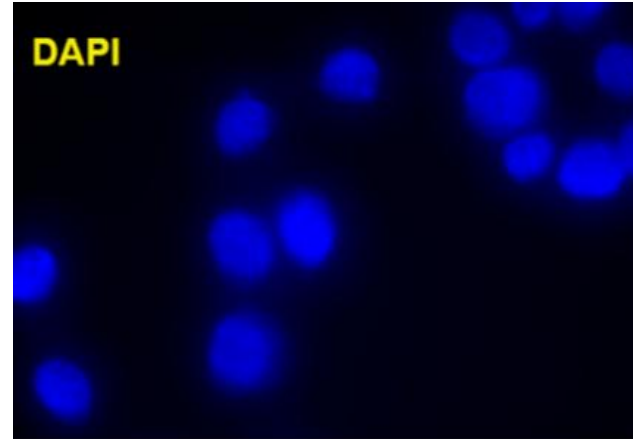
# University of Arkansas and genome-wide gene expression IV



# Progesterone signaling

- Progesterone added to some Hormone Growth Promotant mixes in cattle, elevate FE by 20%.
- Progesterone changes physiology and fibre composition phenotype of muscle in pregnant women.
- **Progesterone receptor co-localises to the mitochondria in birds.**

# Progesterone receptor immuno-staining



# Summary of University of Arkansas research

- Panel of 40 genes expressed in muscle that can discriminate the two groups of birds differing in FE
- More efficient birds have paler muscle, a bias towards type II sprint fibres and altered mitochondrial function.
- Progesterone signalling is predicted to be a driver of the difference observed in muscle gene expression.
- Some feeds are high in progesterone-like compounds (yams)

# Molecular mechanisms of feed efficiency in broilers

Bottje et al. *BMC Systems Biology* (2017) 11:29  
DOI 10.1186/s12918-017-0396-2

BMC Systems Biology

RESEARCH ARTICLE

Open Access

## Progesterone signalling in broiler skeletal muscle is associated with divergent feed efficiency



Walter Bottje<sup>1</sup>, Byung-Whi Kong<sup>1</sup>, Antonio Reverter<sup>2</sup>, Ashley J. Waardenberg<sup>2,3</sup>, Kentu Lassiter<sup>1</sup> and Nicholas J. Hudson<sup>4\*</sup>

### Abstract

**Background:** We contrast the *pectoralis* muscle transcriptomes of broilers selected from within a single genetic line expressing divergent feed efficiency (FE) in an effort to improve our understanding of the mechanistic basis of FE.

**Results:** Application of a virtual muscle model to gene expression data pointed to a coordinated reduction in slow twitch muscle isoforms of the contractile apparatus (*MYH15*, *TPM3*, *MYOZ2*, *TNNI1*, *MYL2*, *MYOM3*, *CSR3P*, *TNNT2*), consistent with diminishment in associated slow machinery (myoglobin and phospholamban) in the high FE animals. These data are in line with the repeated transition from red slow to white fast muscle fibres observed in agricultural species selected on mass and FE. Surprisingly, we found that the expression of 699 genes encoding the broiler mitoproteome is modestly—but significantly—biased towards the high FE group, suggesting a slightly elevated mitochondrial content. This is contrary to expectation based on the slow muscle isoform data and theoretical physiological capacity arguments. Reassuringly, the extreme 40 most DE genes can successfully cluster the 12 individuals into the appropriate FE treatment group. Functional groups contained in this DE gene list include metabolic proteins (including opposing patterns of *CA3* and *CA4*), mitochondrial proteins (*CKMT1A*), oxidative status (*SEPP1*, *HIG2A*) and cholesterol homeostasis (*APOA1*, *INSIG1*). We applied a differential network method (Regulatory Impact Factors) whose aim is to use patterns of differential co-expression to detect regulatory molecules transcriptionally rewired between the groups. This analysis clearly points to alterations in progesterone



# Q-CAS grant submitted February 2017

- Queensland (Uni Qld colleagues) and Chinese Academy of Science (Dr XiangFeng Kong) collaboration
  - Kemin China (Dr. Bing Guo) are the industrial co-funder
  - Explore the molecular basis of nutritional treatments (betaine and curcumin / essential plant oils) that may mitigate heat stress and improve production under sub-tropical conditions
- One part of the project is to use genome-wide gene expression, analogous to the academic broiler research just described

# Overall summary of broiler research

- There is substantial variation in muscle mitochondrial content in broilers and it associates with performance phenotypes including muscle and fat development.
- Patterns of gene expression indicate more efficient broilers have altered muscle structure and mitochondrial metabolism.

# White striping and wooden breast

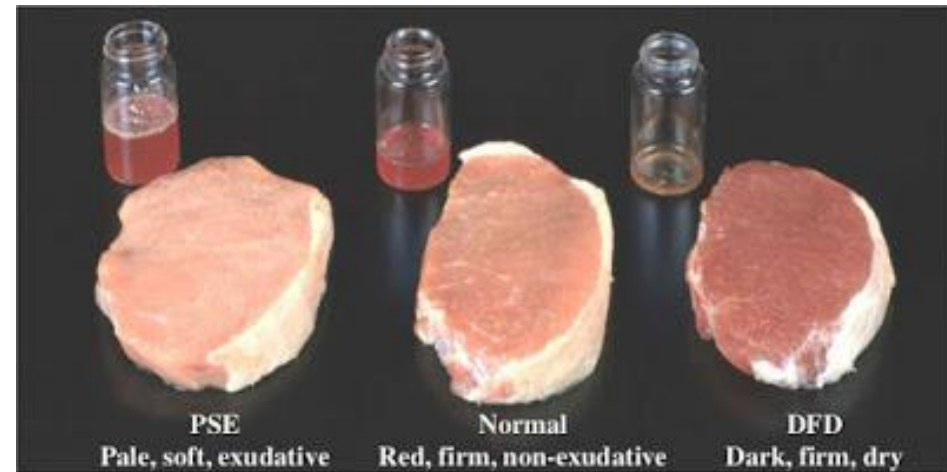
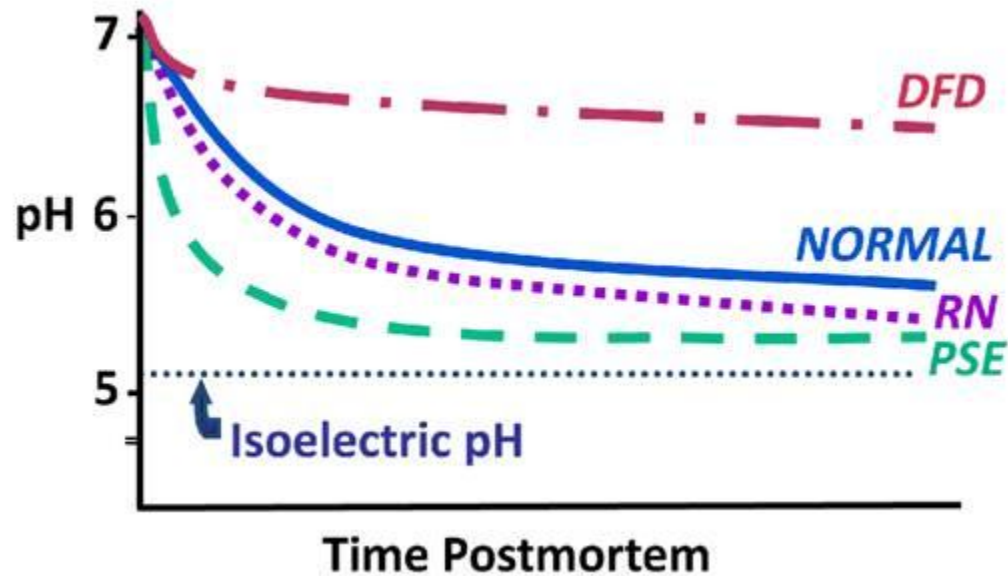


Normal Breast Filet

Moderate White Striping

- Broiler breast muscle has an extreme muscle structure and metabolism
- Lactic acidosis has been observed in live birds predisposed to these conditions, implying inadequate aerobic metabolism
- **Hypothesis:** Is this a consequence of too low a mitochondrial content and capillarity?

# Post mortem pH decline and meat quality

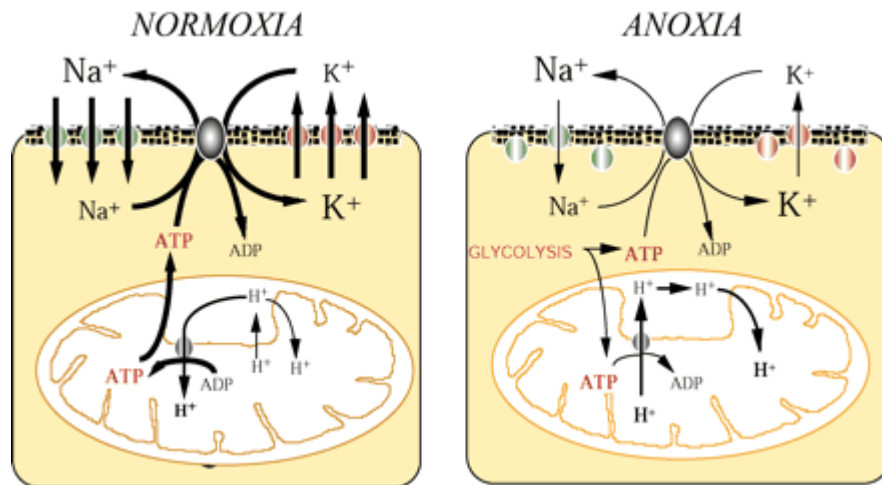


Conventional model relates to stored carbohydrate available for muscle glycolysis and subsequent anaerobic metabolism → pH decline rate and ultimate pH

Given post-slaughter muscle is an enforced anaerobic systems is their a role for the (aerobic) mitochondria?

# Mitochondrial 'treason' during anoxia

- In the absence of oxygen, mitochondria become net consumers rather than producers of ATP
- May hasten anaerobically produced ATP thereby influencing the pH decline curve



# Conclusions

- Mitochondrial content varies across individual broilers and is associated with commercial performance traits
- Patterns of gene expression indicate high FE broilers have paler muscle and altered mitochondrial metabolism
- Mitochondrial phenotypes are responsive to environmental cues (ambient temperature, exercise and diet).
- Should the Q-CAS grant (co-funded by Kemin) be funded we have the opportunity to explore some of these ideas in the context of nutritional manipulation of pig production under heat stress

# Acknowledgments

- Cobb Vantress (Rachel Hawken and Ron Okimoto)
- University of Arkansas (Walter Bottje and Whi Kong)
- CSIRO (Toni Reverter)
- Colleagues at Uni Qld (Judy Cawdell-Smith, John Gaughan, Elham Soumeh, Wayne Bryden), Kemin China (Bing Guo) and CAS (Xiang Feng Kong)
- Kemin Nutrition 2017 organising committee