

Alteration of mtDNA copy number, mitochondria-related gene expression and metabolites in grass-fed and grain-fed Angus cattle

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Introduction

- Grass-fed and grain-fed beef cattle differ in diet regimes. Different dietary sources have been suggested to have different effects on mitochondrial function and dynamic behavior.
- In response to changes in energy demand and supply, the organism regulates mitochondrial metabolic status to coordinate ATP production, which would significantly influence whole body metabolism and gene expression.
- We initially explored the mtDNA copy number, mitochondria-related gene expression, and metabolic biomarkers in grass-fed and grain-fed Angus beef cattle.

Materials and Methods

Animals and samples

Animals: grass-fed, grain-fed beef cattle are from Wye Angus Farm. Tissue: Muscle, Liver, Spleen, Rumen

mtDNA copy number analysis

mtDNA copy number was detected using qPCR. *ACTN* and *ND2*, *ND5*, *CYTB*, *COX3*, *12sRNA* were used as the nuclear reference gene and mitochondrial genome gene.

RNA sequencing and analysis

Raw RNA-seq data from the previous report. Raw data were processed using Perl scripts to remove reads containing adapter, poly-N and low quality. The remaining reads were mapped to the bovine genome (ARS-UCD 1.2). A gene was considered to be differentially expressed when $FDR < 0.15$.

Metabolomics profiling

LC/MS, GC/MS, QA/QC. Tissue: muscle and blood. The MUVR package in R was used to find the metabolic biomarkers.

Discussion

- The grass-fed group had higher mtDNA copy number than grain-fed group.
- *COX6A2*, *POLG2*, *PPIF*, *DCN*, and *NDUFA12*, involving in ATP synthesis, mitochondrial replication, transcription and maintenance, might contribute to the alterations of mtDNA copy number and gene expression.
- Integrated analysis of the altered metabolites and gene expression revealed *MDHI* might contribute to the mitochondrial NADH oxidation and spermidine metabolism in grass-fed and grain-fed cattle.

Results

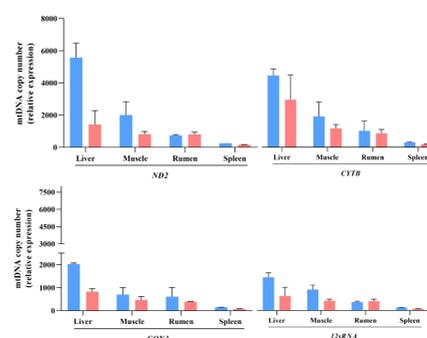


Figure 1. The mtDNA copy number variation in different tissues between grass-fed and grain-fed.

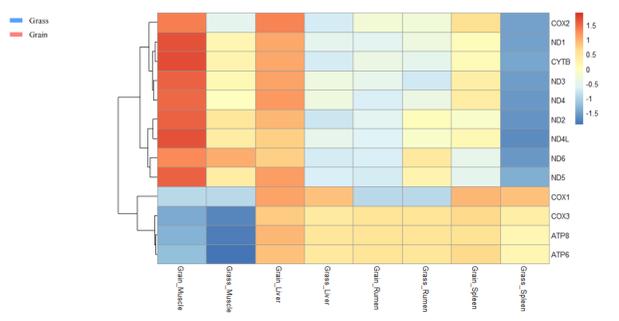


Figure 2. Heat map showing relative expression of mtDNA encoded genes in the four tissues of grass-fed and grain-fed Angus cattle.

Table 1: Differentially expressed mitochondria-related gene in the four tissues

Tissue	Comparison	MtDNA-encoded gene		Mitochondria-related gene	
		Up	Down	Up	Down
Muscle	Grass vs Grain		<i>ND1, ND2, ND3, ND4, ND5, COX2, CYTB</i>	<i>CKMT2, UBB, MDH1</i>	<i>ACSM2B, HAO2, PRSS35, CLU1, APOPT1, ALDH6A1, FKBP8, MRPL40, COX6A2, BCL2L1, CLPP, HSP90AA1</i>
				<i>CAT, ACOT13, NDUFA1, CYP2E1, NT5DC3, TMLHE, FAM210A, KMO, PCK2, UBB, PNPT1, MGST1, DHCR24, SOD1, TMBIM6, POLG2, MPCI, ALDH9A1, GLUD1, ALDH2, SHMTL, ALDH1A1, GATM, SORD, PCCB, HAGH, SOD2</i>	
Liver	Grass vs Grain		<i>ND1, ND2, ND5, COX2, CYTB</i>		
Rumen				<i>CKMT1A, SHMT1, CBR3, SLC25A10, TSPO, ETHE1, DHCR24, AK2, PPIF, GSR, GLYCK</i>	<i>COX7B2, MRPL33, COX4I2, DCN</i>
Spleen			<i>ND3</i>	<i>DNAJC19, BDH1, NDUFA12, MGARP, HADH, ATP5F1B, MTHFD2L</i>	<i>LYRM7, CYP11A1</i>

Table 2. Top five Gene Ontology terms in the four tissues.

Tissue	GO term_Biological Process	FDR
Muscle	cellular respiration	1.51E-11
	oxidation-reduction process	4.18E-11
	ATP synthesis coupled electron transport	8.73E-10
	nucleobase-containing small molecule metabolic process	5.99E-09
	ATP metabolic process	5.28E-08
Liver	oxidation-reduction process	3.08E-17
	small molecule metabolic process	1.32E-13
	metabolic process	9.41E-11
Spleen	cellular metabolic process	9.41E-11
	cellular process	1.04E-08
	oxidation-reduction process	0.0117
Rumen	positive regulation of mitochondrion organization	0.0356
	cellular metabolic process	0.0356
	organonitrogen compound metabolic process	0.0356
	cellular modified amino acid metabolic process	0.0419
	phosphorylation	0.0419

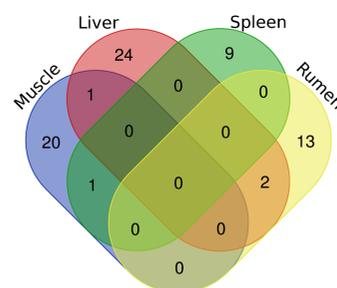


Figure 3. Venn diagram showing the number of DEGs commonly expressed in the four tissues.

Pathway	Sub-pathway	Biochemical Name	B	M
Amino Acid	Arginine and Proline Metabolism	N-methylproline		
		1-eicosapentaenoylglycerophosphoethanolamine		
Lipid	Lysolipid	2-eicosapentaenoylglycerophosphoethanolamine		
		1-linolenoylglycerophosphocholine (18:3n3)		
		3-dehydrocarnitine		
Xenobiotics	Food Component/Plant	homostachydrine		

Figure 4. Differentially regulated metabolites and metabolic pathways overlapped in blood and muscle of grain-fed group compared to the grass-fed group. B: blood; M: muscle.

Conclusions

- The results provide a basis to further elucidate the adaptive and regulatory modulation of the mitochondrial function in response to different feeding systems in Angus cattle.

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