Project/Activity Number: Multistate Research Project NC1184
Project/Activity Title: Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation
Period Covered: 10/2016 to 9/2017
Date of This Report: 11/07/2017
Annual Meeting Dates: 10/20-21/2017

Station Participants:

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Summary of Minutes – Annual Meeting:

The annual NC1184 technical committee meeting was held on October 20 and 21, 2017, at the Straughn Professional Development Center located in Gainesville, Florida, on the University of Florida campus; it was hosted by Dr. Tracy Scheffler of the Department of Animal Science, Florida State University. On October 20th, Dr. Geoff Dahl, Department Head of Animal Science, welcomed the group and shared information about the department and the university. The group then began with oral station reports. A lunch break (kindly funded by Dr. Dahl) was provided. After lunch, the group continued with more station reports. In the evening, the group met for dinner, which was hosted by Dr. Tracy Scheffler at the University of Florida Beef Teaching Unit. The following morning the group started with a conference call with Dr. Mark Mirando (USDA-NIFA), who outlined the current funding programs, budgets, and statistics on the number of proposals submitted annually and funding rates. Following a question and answer session with Dr. Mirando, the remaining oral station reports were given. Following the station reports, the group discussed where the meeting would be held next year and it was decided that Dr. Tracy Scheffler would send an email out to the whole group as there were relatively few attendees at this year's meeting. After that email was sent, it was decided that Dr. Derris Burnett, of the Mississippi station, would host in 2019.

Accomplishments:

Objective 1: ***Characterize the signal transduction pathway that regulates skeletal muscle growth and metabolism including the influence of endogenous growth factors and various production practices.***

Alabama Station:

1. Characterization of myogenic stem cell heterogeneity and fiber morphometrics in two divergently selected broiler chicken lines.
    a. Completed the live animal, sample collection, and data collection portions of the project.
    b. Presented an abstract at the 2017 Poultry Science Association annual meeting.
2. Impact of in ovo thermal manipulation on broiler chicken muscle development, growth, and satellite cell activity.
    a. Completed the live animal growth performance and carcass yield data collection and sample collection portions of the project.
    b. The cryohistology and immunofluorescence analysis portions of the project are ongoing.
3. Effects of dietary amino acid density on growth performance, satellite cell activity, collagen gene expression, and the incidence of wooden breast.
    a. Completed the live animal growth performance and carcass yield data collection, sample collection, and data analysis portions of the project.
    b. Presented an abstract at the 2017 Poultry Science Association annual meeting.
    c. Submitted manuscript to a peer-reviewed journal, *Poultry Science*

Connecticut Station:

1. Effects of poor maternal nutrition during gestation on fetal muscle development
    a. Completed immunohistochemistry of fetal muscle samples at d45, d90, d135, and birth, determined muscle fiber CSA of primary and secondary fibers at d90, determined number of Pax7(+) progenitor cells at all four time points.
    b. Completed metabolome analysis of longissimus dorsi muscle from offspring of over-, restricted-, and control-fed ewes at day 45, 90, 135 of gestation and within 24 h of birth.
    c. Identified changes in lipid peroxidation as a result of maternal diet in serum and muscle in offspring within 24 h of birth.

Hawaii Station:

1. Myostatin inhibitory region of pig myostatin propeptide
    a. **Background:** Myostatin (MSTN) is a potent negative regulator of skeletal muscle growth, and its activity is suppressed by MSTN propeptide (MSTNpro), the N-terminal part of MSTN precursor cleaved during post-translational processing. We have previously shown that bioactive pig MSTNpro could be produced in an *E. coli* system. The current study examined which region of pig MSTNpro is critical for MSTN inhibition.
    b. **Method:** Four truncated forms of pig MSTNpro containing N-terminal maltose binding protein (MBP) as a fusion partner were expressed in *E. coli*, and purified by

affinity chromatography. The MSTN-inhibitory capacities of these proteins were examined by the pGL3-$(CAGA)_{12}$ luciferase reporter assay.

c. **Results:** A MBP-fused, truncated MSTNpro containing residues 42-175 (MBP-Pro42-175) exhibited the same MSTN-inhibitory potency as the full sequence MSTNpro. Truncated MSTNpro proteins containing either residues 42-115 (MBP-Pro42-115) or 42-98 (MBP-Pro42-98) also exhibited MSTN-inhibitory capacity with lower potencies than that of full sequence MSTNpro. Removal of MBP from MBP-Pro42-175 and MBP-Pro42-98 resulted in 20-fold decrease in MSTN-inhibitory capacity of Pro42-175 and abolition of MSTN-inhibitory capacity of Pro42-98, indicating that MBP as fusion partner enhanced the MSTN-inhibitory capacity of those truncated MSTNpro proteins. Interestingly, IC_{50} value of MBP-Pro42-175 for MSTN inhibition was almost 4-fold lower than that for GDF-11 inhibition, while IC_{50} value of full sequence MSTNpro for MSTN inhibition was not different from that for GDF-11 inhibition, indicating that MBP-Pro42-175 specifically inhibits MSTN with less cross-reactivity to GDF-11.

Idaho Station

1. Completing an aquaculture feeding trial in *sablefish* (Anoplopoma fimbria) examining the influence of rearing temperature and dietary composition on growth traits and temporal expression of myogenic and metabolic genes in both white and red skeletal muscle. This comprises the final research trial for an MS student with projected completion of December 2017.

Illinois Station:

1. Interaction of IGF2 and Myostatin in the Regulation of Muscle Growth and Development
    a. Pigs, heterozygous for a novel inactivating mutation of myostatin, were raised to market weight and characterized. These pigs had either the favorable IGF2 paternal A allele or the unfavorable paternal G allele (IGF G3072A). Live weights were similar between all genotypes (average 124kg) but weights of individual muscles were increased 10-30% in myostatin heterozygous pigs compared with wild type pigs. The effects of the favorable IGF2 allele and the myostatin mutation were completely additive.
    b. Mice were genetically edited using tail-effector-like endonuclease technology (TALEN) to mimic the naturally occurring IGF2 G3072A mutation. This resulted in three distinct lines of mice possessing the desire G>A point mutation only, the desired G>A point mutation with an additional A>G mutation at the target site, and C-insertion mutation. All mutations prevented the binding of a transcriptional repressor ZBED6. Preliminary data from characterization of these mice indicate that body weight and muscle weights are increased in all three mutated lines compared with wild type mice. However, in contrast to the similar mutation in pigs, this increase in body weight does not appear to be restricted to muscle. Organ weight and adipose tissue weight were also increased in these mutated mice.

Indiana Station:

1. Pten is a phosphatase that antagonize growth factor (IGF1) signaling. We reported deletion of Pten in embryonic myoblasts leads to postnatal muscle hypertrophy but disrupts satellite cell homeostasis (Yue et al, 2016, *Cell Reports*).

Iowa Station:

1. We completed tissue collection for our next experiment related to modification of the PGC-1α pathway via nutraceuticals. We have expanded our interventions to include quercetin, nicotinamide riboside, Lisinopril, and Prednisone and combinations thereof. Preliminary analyses are underway and a histological and biochemical examination will begin soon.
2. We confirmed dysfunctional autophagy in dystrophic skeletal muscle. Importantly, we are the first group to document release of autophagosomes, termed autophagosome escape, from dystrophic muscle. Importantly, we also collected compelling evidence of this same phenomenon occurring in healthy muscle. As they are found in the extracellular space they may participate in paracrine signaling and considering the mass of muscle and that they escape the muscle environment may participate in endocrine signaling.
3. We have begun experiments to better understand why autophagy is dysfunctional in dystrophic muscle, which are now focused on regulation of transcription factor EB, the primary transcription factor driving lysosome biogenesis.

Minnesota Station:

1. Protein synthesis was evaluated in equine satellite cell myotube cultures treated with a leucine titration ranging from 0- to 408-μ*M*. Our results show a 1.8-fold increase ($P < 0.02$) in protein synthesis at levels slightly greater than those found in the general circulation, 204- and 408-μ*M* when compared to a no leucine control (0-μ*M*). Puromycin incorporation, a nonradioactive surface sensing of translation (SUnSET) methodology, demonstrated a 180% increase ($P = 0.0056$) in puromycin incorporation in leucine compared to control cultures.
2. When equine satellite cell myotube cultures were treated with leucine (LEU; 408-μM) or a no-leucine control (CON) in the presence or absence of rapamycin (LR and CR, respectively), an inhibitor of mTOR, rapamycin, suppressed phosphorylation of mTOR ($P < 0.01$) and rS6 ($P < 0.01$) with an increase in phosphorylation of rS6 in leucine-treated cultures observed when compared to control cultures ($P < 0.05$). Similarly, there was a 27% increase ($P < 0.005$) in the hyperphosphorylated γ-form of 4E-BP1 compared to total 4E-BP1 in LEU compared to CON cultures with leucine-induced phosphorylation of 4E-BP1 completely blocked by rapamycin with a smaller decrease observed in CR compared to CON cultures.

Mississippi Station:

1. The effects of dietary lysine level on the blood plasma concentrations of protein, carbohydrate, and lipid metabolites were investigated in late-stage finishing pigs.
    a. The 3 dietary lysine levels were 0.43% (a deficient level; for Diet I), 0.71% (an adequate level; for Diet II), and 0.98% (an excess level, for Diet III).
    b. There were no differences ($P > 0.10$) between pigs fed Diets II and III in the plasma concentrations of urea nitrogen, albumin, and total cholesterol, the concentration of albumin was higher ($P < 0.05$) than that of pigs fed Diet I, and

the concentrations of urea nitrogen and total cholesterol were lower ($P < 0.05$) than that of pigs fed Diet I.
   c. There were no differences in plasma insulin and GH concentrations ($P > 0.05$) between the three dietary treatments.
   d. Plasma IGF-1 concentration of the pigs fed either Diet I or Diet II was lower ($P < 0.05$) than that of the pigs fed Diet II.
   e. There were no differences ($P > 0.10$) among the three dietary treatments in the plasma concentrations of total protein, triglycerides, and glucose.

New Jersey Station:

1. Delineated the effects of acute strenuous exercise, as well as a 12-week exercise-training program, on plasma amino acids and the skeletal muscle metabolome in mature, Standardbred horses. These measurements revealed fitness to differentially alter lipid metabolism, branched-chain amino acid metabolism and nucleotide metabolism following acute exercise. Expression (mRNA levels) of the transcription factor ATF4 and other genes known to regulate metabolism are currently being measured in the same skeletal muscle samples. This work is under manuscript preparation and thesis preparation.
2. Examined long term control of muscle protein synthesis in mice fed a sulfur amino acid restricted diet. Results show that dietary sulfur amino acid restriction reduces muscle protein synthesis. Genetic deletion of the amino acid sensor GCN2 in mice did not rescue this decline in skeletal muscle protein synthesis.

Utah Station:

1. Determination of mechanism through which decreased plane of nutrition in second trimester alters end-product quality of offspring in beef cattle
   a. Samples were collected from offspring of mother cows that either maintained BCS during the second trimester (MAIN) or from cows that dropped one BCS during the second trimester of pregnancy. Samples were collected from the *longissimus dorsi* at weaning, prior to beginning the feedlot phase and immediately following harvest.
   b. Completed miRNA analysis of samples from weaning, the beginning of the feedlot phase and immediately following harvest. Ten different miRNA were analyzed using qRT-PCR methods.
2. Gained insight into how different organic pastures impact dairy heifer development.
   a. A total of 6 animals per treatment were used to study 8 different pastures.
   b. Animal growth, serum IGF-1 concentration, blood urea nitrogen concentration, and parasite load were measured.

Washington Station:

1. During the past years, we have been focused on the impacts of maternal nutrition on the early development of skeletal muscle and brown adipose tissue.
2. We also explored the role of AMPK in regulating brown adipose tissue development.
   a. We found that AMPK regulates brown adipogenesis through providing a key metabolite, alpha-ketoglutarate, which is required for DNA demethylation in the Prdm16 promoter and brown adipogenesis.
3. In addition, we are also defining the role of vitamin A and its metabolite, retinoic acid, in mediating adipose tissue development in beef cattle.

Wyoming Station:

1. RBM20 regulates splicing essential for myofiber structure and skeletal muscle physiology.
    a. Completed animal experiments. 3 months and 6 months old WT and RBM20 KO male Sprague-Dawley rats are used to study the differences in myofiber structure and skeletal muscle physiology. Skeletal muscles (longissimus dorsi, soleus, extensor digitorum longus, tibialis anterior and gastrocnemius) were collected. The tissues were snap-frozen in liquid nitrogen and stored at -80 celsius degree or embedded in OCT medium for cryosectioning.
    b. Completed the study of RBM20 expression in different types of skeletal mucle (longissimus dorsi, soleus, extensor digitorum longus, tibialis anterior and gastrocnemius).
    c. Completed myofiber structural and physiological analysis in skeletal muscle. We studied the change in muscle mass and myofiber cross sectional area between WT and RBM20 KO rat skeletal muscles (soleus, extensor digitorum longus, tibialis anterior). In addition, we studied fibrosis development and sarcolemma integrity in these skeletal muscles.
    d. Completed myofilament protein analysis in skeletal muscles. We investigated myosin heavy chain type distribution in WT and RBM20 KO rat skeletal muscles (soleus, extensor digitorum longus, tibialis anterior).
2. Effects of maternal obesity (MO) during gestation on fetal muscle function and development
    a. Completed the molecular mechanism study of the effect of MO on cardiac muscle contractility and calcium insensitivity in offspring of MO ewes. We found that MO reduced expression of myosin heavy chain in fetal myocardium. Moreover, the cardiac troponin T and troponin I expression level was upregulated in fetuses from obese mothers, whereas MO downregulated the expression of troponin C in fetal myocardium. Also, we found that MO increased the phosphorylation of protein kinase A (PKA) as well as the phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which further promoted phosphorylation level of ryanodine receptor type 2 (Ryr2). Increased CaMKII and PKA phosphorylated Ryr2 plays a critical role in the development of MO-induced sarcoplasmic reticulum Ca^{2+} leak in fetal cardiomyocytes.
    b. Elevated cortisol level in offspring of obese ewes induces autophagic gene up-regulation in heart muscle. We found that the autophagic cargo protein SQSTM1, also known as ubiquitin-binding protein p62, is upregulated in fetal heart from obese ewes, and RNA binding motif 39 (RBM39), a transcriptional factor is activated by cortisol stimulation. our studies reveal autophagy pathways have been activated via RBM39 transcriptional regulation in fetal myocardium of obese ewes, as well as the autophagy cargo protein SQSTM1.

Objective 2: ***Characterize the cellular and molecular basis of myogenesis***

Alabama Station:

1. Characterization of myogenic stem cell heterogeneity and fiber morphometrics in two divergently selected broiler chicken lines.
    a. Completed the live animal, sample collection, and data collection portions of the project.

    b. Presented an abstract at the 2017 Poultry Science Association annual meeting.
2. Impact of in ovo thermal manipulation on broiler chicken muscle development, growth, and satellite cell activity.
    a. Completed the live animal growth performance and carcass yield data collection and sample collection portions of the project.
    b. The cryohistology and immunofluorescence analysis portions of the project are ongoing.
3. Effects of dietary amino acid density on growth performance, satellite cell activity, collagen gene expression, and the incidence of wooden breast.
    a. Completed the live animal growth performance and carcass yield data collection, sample collection, and data analysis portions of the project.
    b. Presented an abstract at the 2017 Poultry Science Association annual meeting.
    c. Submitted manuscript to a peer-reviewed journal, *Poultry Science*

Arkansas Station:

1. Myogenesis and adipogenesis in muscle is affected by n-3 fatty acids supplement for cell culture
    a. Preliminary studies were conducted using a murine *in vitro* myoblasts model (C_2C_{12}) to detect the change of mitochondria biosynthesis, function, and pathways of adipogenesis caused by n-3 fatty acid (EPA and DHA) treatment.
    b. EPA and DHA were added into culture media to mimic the maternal over-nutrition during gestation.
    c. In the myogenesis, data show fatty acids treatment limits the formation of myotubes, as well as the marker genes expression of myogenesis. Genes expression related to adipogenesis are upregulated by fatty acids treatment. Mitochondrial biosynthesis is inhibited by fatty acids, and it is confirmed by analyzing mitochondrial respiration, which shows fatty acids treatment decreases the oxygen consumption rate. Peroxisomes biosynthesis genes were upregulated by fatty acids.
    d. In white adipogenesis, key genes related to mitochondrial synthesis and metabolism (especially those related to complexes enzymatic activities) were significantly down-regulated by n-3 fatty acids treatment. While during brown adipogenesis, n-3 fatty acids treatment improves ATP synthase, mitochondrial biogenesis, and brown adipogenesis up-stream regulators gene expression.
2. Gene edition technology in transgenic beef production
    a. Galactose-α-1,3-galactose (Alpha-Gal) is a mammalian carbohydrate compound that presents in vibrate animals except humans or Old World monkeys. Alpha-Gal is synthesized by a glycosylation enzyme α-1,3-Galactosyltransferase (GGTA1).
    b. Genetically knocking out GGTA1 in pig protects xenotransplantation from hyperacute rejection
    c. We have designed target gRNAs for the bovine GGTA1 gene. Next step is using CRISPR-Cas9 to edit GGTA1 gene in bovine primary cell culture.
    d. We used the gRNA design tool and selected 5'-GGCCTGACGGTTTTCGCCGT-3' as the target gRNA sequence from the coding DNA sequence of Bos taurus alpha-galactosyltransferase 1 (glycoprotein). The gRNA was constructed in the pSpCas9 BB-2A-GFP (PX458) vector provided by GenScript USA Inc. Vectors were amplified and transfected into BAOSMC by GenePORTER2 transfection reagent

when the cells were 80% confluency. Green fluorescent can be viewed after 24 hours transfection. The transfection efficiency can reach about 70% to 80%.

e. Cells were collected in PBS at pH7.4 after 24 hours transfection. Total protein was extracted then the enzyme-linked immunosorbent assay was used to examine the GGTA1 production. By normalized with the total protein concentration, the GGTA1 protein level in the transfected cells was 17.9 ± 7.25% lower ($P < 0.05$) than in the control cells, showing a significant inhibition of GGTA1 gene expression in the cells by CRISPR-Cas9 gene edition method.
f. Our preliminary data shows that the gRNA sequence that we chose was suitable for the GGTA1 gene knockout in bovine aortic smooth muscle cells. Moreover, the CRISPR-Cas9 system was proved can be applied in the genome editing of bovine cells.

Illinois Station:

1. Influence of maternal infection or oxidative stress on muscle development and epigenetic programming of pigs
    a. During mid-gestation, pregnant sows were inoculated with porcine respiratory and reproductive virus (PRRS) and piglets from these sows were compared with those born of non-infected dams. Muscle cell number in the semitendinosus muscle was reduced 30% in piglets from infected dams. Methylation of analysis of longissimus dorsi muscle of newborn piglets from infected sows revealed differential methylation patterns resulting from maternal infection. Hypermethylation was present in several disease response pathways, in the thyroid hormone and oxidative phosphorylation pathways and in genes involved in fast muscle fiber phenotypes. Coupled with an increase in oxidative myosin heavy chain fiber type gene expression in these animals, these data suggest that maternal infection may limit muscle development and shift muscle fiber types to a more slow, oxidative phenotype.
    b. Beginning at 30 d of pregnancy and continuing until parturition, oxidative stress was induced in sows by feeding soybean oil that had been cooked at 90C for 72 hours. Piglet viability, growth, and muscle development were analyzed and compared with piglets from sows fed fresh oil. Piglets born from sows fed heated oil had reduced heart, lung, and liver weights, and reduced small intestine length compared with piglets born from mothers fed fresh oil. Muscle weight, however, was not affected by oxidative stress. Immunophenotyping revealed sows fed oxidized oil had an approximate 11% reduction in total T-cell population, with an increase in CD4+CD8+ double positive T-cells, compared with sows fed fresh oil.

Indiana Station:

1. We demonstrated that the transcriptional factor Ascl2 is transiently expressed in a subpopulation of embryonic myoblasts to promote the generation of satellite cells during development. Ascl2 achieves this role through inhibiting the transcriptional activity of myogenic regulatory factors (Wang et al, 2017a, *Development*).
2. We used CRISPR/CAS9-mediated gene targeting to demonstrate that loss of MyoD promotes fate transdifferentiation of myoblasts into brown adipocytes (Wang et al 2017b, *ebiomedicine*).

3. We discovered that deletion of PTEN in satellite cells leads to their rapid depletion due to premature differentiation. Furthermore, we found that PTEN interacts with Notch signaling to maintain satellite cell quiescence (Yue et al, 2017 *Nature Communications*).
4. Using conditional knockout mouse models, we demonstrate that the hypoxia inducible factors HIF1α and HIF2α are dispensable for embryonic muscle development, but essential for postnatal muscle regeneration (Yang et al, 2017, *JBC*).

Michigan Station:

1. We studied the effects of thermal challenge on growth of turkey satellite cells

Mississippi Station:

1. The effects of dietary lysine level on the skeletal muscle gene expression were investigated in late-stage finishing pigs using transcriptomic microarray analysis.
    a. The 3 dietary lysine levels were 0.43% (a deficient level; for Diet I), 0.71% (an adequate level; for Diet II), and 0.98% (an excess level, for Diet III).
    b. The results revealed that 674 transcripts were differentially expressed (at $P \leq 0.05$ level). At the $P \leq 0.01$ level, 60 out of 131 transcripts were annotated in the NetAffx database.
    c. Ingenuity pathway analysis showed that dietary lysine deficiency may lead to: (1) increased muscle protein degradation via the ubiquitination pathway as indicated by the up-regulated DNAJA1, HSP90AB1 and UBE2B mRNA; (2) reduced muscle protein synthesis via the up-regulated RND3 and ZIC1 mRNA; (3) increased serine and glycine synthesis via the up-regulated PHGDH and PSPH mRNA; and (4) increased lipid accumulation via the up-regulated ME1, SCD, and CIDEC mRNA.
    d. Dietary lysine excess may lead to: (1) decreased muscle protein degradation via the down-regulated DNAJA1, HSP90AA1, HSPH1, and UBE2D3 mRNA; and (2) reduced lipid biosynthesis via the down-regulated CFD and ME1 mRNA.
2. Completed sample collection on study investigating the effects of melatonin supplementation on fetal porcine muscle development. Pregnant sows were supplemented with (n = 6) or without (n = 6) melatonin 920mg/hd/d) from 30-90 days of gestation at which point they were harvested to collect the developing fetuses for morphological and histological analyses. These offspring were necropsied and the semitendinosus and longissimus dorsi muscles were collected for subsequent analysis.
    a. Immunohistochemistry of fetal muscle samples to determine muscle fiber CSA and fiber type distribution is underway.
    b. Quantitative PCR for expression of mRNA and miRNA involved in muscle growth and development are underway.

North Carolina Station:

1. Increased myostatin expression, resulting in muscle loss, has been associated with hyperammonemia in mammalian models of cirrhosis.
    a. However, there is evidence that hyperammonemia in avian embryos results in a reduction of myostatin expression, suggesting a proliferative myogenic environment.
    b. Increased myostatin expression, resulting in muscle loss, has been associated with hyperammonemia in mammalian models of cirrhosis. However, there is evidence that

hyperammonemia in avian embryos results in a reduction of myostatin expression, suggesting a proliferative myogenic environment.

c. The present in vitro study examines species differences in myotube and liver cell response to ammonia using avian and murine-derived cells.
d. Relative expression of myostatin mRNA, determined by quantitative real-time PCR, was significantly increased in AA (10 mM) treated C2C12 myotubes compared to both ages of chick embryonic myotube cultures after 48 h ($P < 0.02$). Western blot analysis of myostatin protein confirmed an increase in myostatin expression in AA-treated C2C12 myotubes compared to the sodium acetate (SA) controls, while myostatin expression was decreased in the chick embryonic myotube cultures when treated with AA.
e. Myotube diameter was significantly decreased in AA-treated C2C12 myotubes compared to controls, while avian myotube diameter increased with AA treatment ($P < 0.001$). There were no significant differences between avian and murine liver cell viability, assessed using 2', 7'- bis-(2-carboxyethyl)-5-(and-6-)-carboxyfluorescein, acetoxymethyl ester, when treated with AA. However, after 24 h, AA-treated avian myotubes showed a significant increase in cell viability compared to the C2C12 myotubes ($P < 0.05$).

Ohio Station:

1. Effect of Thermal Stress on In Vivo Breast Muscle Growth and Development in Broilers: Association of Wooden Breast with collagen crosslinking
    a. Poultry selected for growth have an inefficient thermoregulatory system and are more sensitive to temperature extremes.
    b. Satellite cells are precursors to skeletal muscle and mediate all posthatch muscle growth. Their physiological functions are affected by temperature.
    c. The objective of the current study was to elucidate the effects of continuous heat exposure the first 2 wk of age on breast muscle development in broilers
    d. Results showed a high level of sensitivity in the satellite cells during the early posthatch period to chronic heat, leading to impaired myogenicity and increased fat.
    e. Results on Wooden Breast studies have shown the possibility of multiple fibrotic myopathies based on collagen crosslinking. The repair and regeneration of muscle fibers mediated by satellite cells appears to be suppressed

Wyoming Station:

1. RBM20 mediates skeletal muscle regeneration after injury.
    a. Completed animal experiments. 9 weeks old WT and RBM20 KO male Sprague-Dawley rats are used to study skeletal muscle regeneration. Tibialis anterior muscle was injured with the injection of 0.5ml of 1.2% of barium chloride and injured muscles are harvested at 18 h, 3 days, 5days, 7 days, 14 days, 1 month and 2 months post-injury. The other hindlimb tibialis anterior muscle was injected with PBS as control. After harvesting the tissues are liquid nitrogen snap-frozen and stored at -80 celsius degree or embedded in OCT medium for cryosectioning.
    b. Completed RBM20 expression during skeletal muscle regeneration.

c. Completed myofiber cross sectional area analysis during skeletal muscle regeneration.
d. Completed analysis of fibrosis level after recovery.
e. Completed analysis of expression of myogenic transcription factors during skeletal muscle regeneration.

Objective 3: ***Characterize mechanism of protein assembly and degradation in skeletal muscle***

Utah Station:

1. Gained insight into the molecular mechanism responsible for development of beef tenderness during aging.
    a. Samples were collected from the *longissimus dorsi* of steaks immediately post-mortem and also hafter 14 days of aging in 100 samples.Samples were then analyzed for tenderness with WBSF methods.
    b. Protein expression of HSPβ1, HSP70, and PARK7 is currently being analyzed for all 100 samples at two different sample collection time points.
2. Increased our understanding of how beta-agonist and implant administrations alters tenderness.
    a. Steaks were collected from the LL of feedlot heifers that received one of three different treatments (n = 11 per treatment) during the feedlot phase: no anabolic implant or beta-agonist (CON), anabolic implant but no beta-agonist (IMP), or an anabolic implant and a beta-agonist (COMBO)
    b. Steaks were aged for 3, 7, 14, 21 or 35 d and samples were analyzed for protein expression of HSPβ1 and HSP70.

<u>Impact Statements:</u>

1. Committee members gained insight into how various environmental factors, such as diet and temperature, impact skeletal muscle growth in chicken, turkeys, sablefish, and cattle. Additionally, we were able to increase our understanding of how supplemental amino acids, nutraceuticals, and vitamins alters metabolism within both the skeletal muscle and adipose of horses, mice, pigs and cattle. Furthermore, several committee members increased our current knowledge regarding the effects that different maternal environments have on skeletal muscle growth of the resultant offspring. These results provide novel information that could be used in several different contexts; (1) improve productivity and quality of meat products from meat producing animals, (2) improve growth of skeletal muscle in both performance animals, such as horses, or in dysfunctional skeletal muscle, such as during muscular dystrophy, and (3) improve our management practice in poultry, dairy, and beef cattle in order to increase overall productivity. Taken together, the new knowledge that was gained from each of the committee members will have an economic impact on livestock production as well as an impact on skeletal muscle health in humans and other companion animals.
2. Committee members investigated different signaling pathways that are responsible for altering the growth of skeletal muscle at a molecular level in several different species, including mice, rats, pigs, cattle, and poultry. An improved understanding of the molecular factors that control skeletal muscle growth will provide new opportunities to further improve skeletal muscle growth and efficiency.

3. Committee members gathered new data to better understand the development of tenderness in meat. New data on the development of tenderness in meat can be used to enhance the quality of beef products that are sold within the United States. Development of a "guaranteed" tender beef product could result in an economic impact of more than a billion dollars.

Collaborative Grants:

1. Collaboration between Michigan and Ohio Station. USDA - National Institute of Food and Agriculture. PD: $975,000. March 15, 2014 - March 14, 2018. Influence of Thermal Challenge on Turkey Muscle Development and Meat Quality. G.M. Strasburg (PD), W.D. Atchison (co-PD), K.M. Reed (co-PD), S.G. Velleman (co-PD).

Grants and Contracts:

1. Auburn University Ag Experiment Station Internal Hatch and Multistate Competitive Funding Program. PD: J. Starkey. Impact of in ovo thermal manipulation on broiler chicken muscle development, growth, and satellite cell activity. 10/01/2015 – 9/30/2017. $50,000
2. Auburn University Ag Experiment Station Internal Hatch Competitive Funding Program. PD: J. Starkey, Co-PD: W.A. Dozier and T. Brandebourg. Effects of dietary amino acid density on growth performance, satellite cell activity, collagen gene expression, and the incidence of wooden breast. 10/01/2015 – 9/30/2017. $50,000
3. USDA/AFRI. PD: K. Govoni, Co-PD: S.A. Reed, S. Zinn, K. Vonnahme. The effects of nutrient restriction and realimentation on offspring liver and muscle growth and metabolism. 01/01/2017-12/31/2021. $489,826.
4. Idaho Beef Council. PI: Gordon Murdoch. RP-histidine supplementation of beef cattle in the feedlot; increasing whole carcass commercial value PI Gordon Murdoch. (July 01,2017-June 30, 2018) $30,000
5. INBRE-NIH. PI Bryn Martin co-PI Nathan Schiele, Gabriel Portirniche and Gordon Murdoch. Investigating the Impact of Arachnoid Trabeculae on Brain Tissue Stresses in Sports-Related Traumatic Brain Injury (TBI)". 03/2017- 03/2019 $75,000/annum.
6. Percussionaire. PI's Tao Xing, Rabijit Dutta and Gordon Murdoch. Comparison of Two Phasitron designs for IPV. 03/2017-07/2017 $12,183
7. Idaho Beef Council. PI: Gordon Murdoch. Year two: Improving color, color stability and flavor of the top sirloin through dietary rumen protected histidine supplementation. July 2016- June 2017 $33,863
8. Idaho Beef Council. PI: Gordon Murdoch. July 2016-June 2017 $23,640
9. Center for Modeling Complex interactions (CMCI)-NIH. PI Tao Xing (Engineering) co-PI Gordon Murdoch. Multi-scale Model of Interaction between Lung and Pulmonary Ventilation PI Tao Xing (Engineering) co-PI Gordon Murdoch. May 2016- April 2017. $108,975 one year with potential for second year renewal
10. USDA-WRAC. PI: Gordon Murdoch. Determination and practical application of egg quality measures towards reliable culture of high-value marine finfish species. FY 2016-2019 Extension Amount: $66,960

11. Hatch grant. PI: Y Huang. Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation
12. Arkansas Beef Council. PI: Y. Huang. Production of α-1,3-Galactosyltransferase gene-deficient in bovine primary cell culture. 2017-2019. $30,000
13. University of Illinois Division of Nutritional Sciences Vision 20/20. PI: A. Dilger. Co-PI: M. Overholt, R. Dilger. Effects of oxidized oil consumption during gestation on sow and piglet oxidative and inflammatory status and piglet brain and muscle development $19,765. October 2016-September 2018. $19,675
14. University of Illinois Campus Research Board. PI: A. Dilger. Co-PI: J. Beever. Effects of transgenerational epigenetic modification on the viability of myostatin gene-edited pigs. May 2015-May 2016. $19,920.
15. 1 R01DK109714-01A1 (MPI: Anthony/Wek). PI: T. Anthony. Homeostatic Responses to Amino Acid Insufficiency. 09/20/16 – 06/30/21 . 2 sum mo.NIH/NIDDK. $2,730,392.00 total funding
16. 1 R01 DK105032. NIH/NIDDK (Morrison). Subcontract PI: T. Anthony. FGF21 is an endocrine signal of protein restriction. 12/01/15 – 11/30/2017. 0 mo. NIH/NIDDK (Morrison). $90,250.00 total funding
17. 2 R01DK096311-05 (Gettys). Subcontract PI: T. Anthony. The major goal of this project is to examine the impact of PERK deletion on the metabolic phenotype associated with dietary methionine restriction. 04/01/2017 – 03/31/2019. 1 sum mo. NIH/NIDDK.131,632 total funding
18. . *NIH (R01HD067449)*. **Du, M.** (PI), and M. J. Zhu. Maternal obesity, AMPK and fetal brown adipogenesis. (8/1/2017-7/31/2022). $ 1,561,745
19. *USDA-AFRI* (2016-68006-24634). **Du, M.** (PI), J. S. Neibergs, D. A. Llewellyn, J. R. Busboom, and M. L. Nelson. High quality beef – a niche market for small and medium sized farms. (1/1/2016-12/31/2019). $479,995
20. *USDA-AFRI* (2016-67015-24470). Jiang Z., **M. Du** (Co-PI), L. K. Fox, and M. Maquivar. Genome wide mapping of alternative polyadenylation sites in cattle. (1/1/2016-12/31/2018). $470,000
21. *USDA-AFRI (*2015-67015-23219). **Du, M.** (PI), J. R. Busboom, and M. L. Nelson. Vitamin A, Zfp423 and intramuscular adipogenesis in beef cattle. (4/1/2015-3/31/2019). $500,000
22. *NIH* (R21AG049976). **Du, M**. (PI). Zfp423 and progenitor adipogenesis during aging. (4/1/2016-3/31/2018). $404,777
23. *NIH* (R15AA024284). Zhang, H., and **M. Du** (Co-PI). Mechanism of chronic alcohol consumption-induced cancer associated cachexia. (01/01/2017-12/31/2020). $456,000.
24. Wyoming Agricultural Experiment Station Funding through USDA/NIFA. PD: W. Guo. Co-PD: S. Ford, J. Ren Molecular Mechanisms Mediating the Effects of Maternal Obesity on Cardiac Function and Development in Fetuses and Offspring of Obese Mothers. 09/01/2015-08/31/2018. $90,000
25. NIH P20 NIGMS 103432. PI: W. Guo. Role of RBM20 in the regulation of cardiac gene splicing in heart failure. 05/01/2016-04/30/2018. $320,000
26. American Heart Association. PI: W. Guo. The role of posttranslational modification of RBM20 in regulating titin isoform transition. 01/01/2016-12/31/2017. $140,000
27. NIH-Wyoming INBRE; NIH P20 NIGMS 103432. Co-PI: W. Guo. Restoring Cardiac Function in Failing RBM20-/- Rats. 5/1/2017 – 4/30/2019. $50,000.

28. USDA-OREI. PD: S.C. Isom CoPD: K.J. Thornton, B. Waldron, A. Young, D. Fuez, E. Creech, K.A. Rood, R. Miller, M. Peel, D. Heleba. Economic and environmental sustainability of heifer development strategies in pasture-based organic dairy systems. 09/17 – 08/20. $999,404
29. WSARE. PD: B. Waldron CoPD: K.J. Thornton, S.C. Isom, A. Young, D. Fuez, E. Creech, K.A. Rood, M. Peel. Grass-birdsfoot trefoil mixtures to improve the economic and environmental sustainability of pasture-based organic dairies in the western U.S. 9/17-8/19. $214,123
30. Utah State Ag. Experiment Station. PI: S.C. Isom, CoPI: K.J. Thornton, B. Waldron, K.A. Rood, A. Young, D. Fuez, E. Creech, M. Peel, R. Miller. Economic and environmental sustainability of heifer development strategies in pasture-based dairy systems. 7/16-6/18. $70,000
31. Simplot Land and Livestock. PD: K.J. Thornton. Determination of the Role of Small Heat Shock Proteins in the Development of Beef Tenderness. 03/17-06/18. $15,000

Publications (peer reviewed journals):

1. Hoffman, M. L., S. A. Reed, S. M. Pillai, A. K. Jones, K. K. McFadden, S. A. Zinn, and K. E. Govoni. The effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism. *J Anim Sci.* 2017. 94:3093-3099. doi:10.2527/jas.2016-1229
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7. K. J. Thornton, K.C. Chapalamadugu, E. M. Eldredge and **G.K. Murdoch** Analysis of Longissimus thoracis protein expression associated with variation in carcass quality grade and marbling of beef cattle raised in the Pacific northwestern United States. (2017) *Journal of Agricultural and Food Chemistry* January 24 DOI 10.1021/acs.jafc.6b02795
8. B. M. Murdoch, **G. K. Murdoch**, S. Greenwood, S. McKay Nutritional Influence on Epigenetic Marks and Effect on Livestock Production. (2016) *Frontiers in Genetics: nutrigenomics*., doi: 10.3389/fgene.2016.00182

9. Chen Y, Wang J, Yang S, Utturkar S, Crodian J, Cummings S, Thimmapuram J, San Miguel P, **Kuang S**, Gribskov M, Plaut K, Casey T. 2017. The Effect of High Fat Diet on Secreted Milk Transcriptome in Mid-lactation Mice. *Physiol Genomics,* in press
10. Xiong Y, Page JC, Narayanan N, Wang C, Jia Z, Yue F, Shi X, Jin W, Hu K, Deng M, Shi R, Shan T, Yang G, **Kuang S***. 2017. Peripheral neuropathy and hindlimb paralysis in a mouse model of adipocyte-specific knockout of *Lkb1*. *eBioMedicine. DOI: http://dx.doi.org/10.1016/j.ebiom.2017.09.017*
11. Jiang C, Cano Vega MA, Yue F, Kuang L, Narayanan N, Uzunalli G, Merkel MP, **Kuang S***, Deng M*. 2017. Dibenzazepine-loaded Nanoparticles Induce Local Browning of White Adipose Tissue to Counteract Obesity. *Mol Ther*. 25(7):1718-29. doi: 10.1016/j.ymthe.2017.05.020.
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Book Chapters:

None

Abstracts, Posters, and Professional Presentations:

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11. Breann N. Sandberg, Carl W. Hunt, Matthew E. Doumit, Ron Richard and **Gordon K. Murdoch** Pacific Northwest Animal Nutrition Conference graduate research competition, January 2017, Richland, WA.
12. E. K. Hogan, J.E. Beever, and A.C. Dilger (2017) The Effects of Virally-Induced Maternal Inflammation on the Methylation Patterns of Skeletal Muscle in Offspring. FASEB Journal 31:979.2
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19. Barnes, N.E.; Strasburg, G.M.; Velleman, S.G.; Reed, K.M. 2017. Transcriptional Response to Thermal Stress in Turkey Muscle. Poster 1134. Plant and Animal Genome Meeting
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23. Presentation: Acute exercise and training distinctively alter branched-chain amino acid metabolic signatures in the skeletal muscle of Standardbred horses. NIDDK Meeting: *Emerging Roles of BCAAs in Human Health and Disease*, May 25-26, 2017.
24. Presentation: School of Health Professions Nutritional Sciences Open Forum, "Dietary Methionine Restriction and the Integrated Stress Response", Rutgers-Newark, June 15, 2017
25. Qiurong Wang, John F. Odhiambo, Chris Pankey, Adel Ghnenis,Peter W. Nathanielsz, Stephen P. Ford, Wei Guo. Molecular Basis of Maternal Obesity Induced Fetal Cardiac Contractile Dysfunction. The AHA scientific meeting, Anaheim, CA. November 11th -15th 2017.
26. Qiurong Wang[1], John F. Odhiambo[1], Peter W. Nathanielsz[1], Stephen P. Ford[1], Jun Ren[2], Wei Guo[1]. RBM39 is Vital for Maternal Obesity-Induced Fetal Sheep Cardiac Contractile dysfunction by Regulating Myocardial Autophagy. The AHA scientific meeting, Anaheim, CA. November 11th -15th 2017.
27. Qiurong Wang[1,2], Chaoqun Zhu[1], John F. Odhiambo[1,2], Guadalupe L Rodríguez-González[3], Peter W. Nathanielsz[1,2], Stephen P. Ford[1,2], Jun Ren[4] and Wei Guo[1,2]. Maternal Obesity Compromises Mitochondrial Bioenergetic Profile of Term Fetal Sheep Heart. 64th SRI annual Scientific meeting, Orlando, FL. March 15th -18th 2017. (Oral Presentation)
28. R.G. Christensen, N.E. Ineck, K.J. Phelps , S.M. Ebarb , J.S. Drouillard , J.M. Gonzalez, K.J. Thornton. Small heat shock protein abundance differs during aging in steaks from the longissimus lumborum of cattle that received different growth promotants. Reciprocal Meats Conference, June 2017, College Station, TX.
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32. Lemire, R. L., K. F. Coble, D. Garry, L. L. Thomas, C. W. Hastad, and D. D. Burnett. 2017. 127 Effect of double stocking and nursery split-out age on wean-to-finish growth performance and economic parameters of growing pigs. J. Anim. Sci. 95(Suppl2):60-60
33. McCarty, K. J., M. P. T. Owen, C. G. Hart, K. C. Yankey, R. C. Thompson, D. D. Burnett, E. H. King, R. M. Hopper, and C. O. Lemley. 2017. 487 Effect of melatonin supplementation during mid- to late-gestation on maternal uterine blood flow and calf size at birth. J. Anim. Sci. 95(Suppl4):238-238.
34. Yang, Z., M. S. Hasan, R. Thompson, M. A. Crenshaw, D. D. Burnett, J. K. Htoo, and **S. F. Liao.** 2017. Effects of dietary methionine deficiency on the growth performance and plasma concentrations of selected metabolites in growing pigs. *J. Anim. Sci.* 95 (Suppl. 4): 43. Abstract was presented by ZY at the ASAS-CSAS Annual Meeting & Trade Show, Baltimore, MD. Jul. 8-12.
35. Hasan, M. S., M. A. Crenshaw, J. M. Feugang, and **S. F. Liao**. 2017. Effects of dietary lysine restriction on the concentrations of free amino acids and other selected metabolites in the blood plasma of growing pigs. *J. Anim. Sci.* 95 (Suppl. 4): 49-50. Abstract was presented by MSH at the ASAS-CSAS Annual Meeting & Trade Show, Baltimore, MD. Jul. 8-12.
36. Wang, T., M. S. Hasan, M. A. Crenshaw, G. Wu, and **S. F. Liao**. 2017. Effects of dietary lysine supply on the plasma concentrations of growth-related hormones in late-stage finishing pigs. *J. Anim. Sci.* 95 (Suppl. 4): 205-206. Abstract was presented by MSH at the ASAS-CSAS Annual Meeting & Trade Show, Baltimore, MD. Jul. 8-12.
37. Humphrey, R. M., Z. Yang, M. S. Hasan, M. A. Crenshaw, D. D. Burnett, and **S. F. Liao**. 2017. The carcass characteristic shift in the compensatorily-gained pigs produced from feeding a methionine-deficient diet. Poster presentation by RMH at the Spring Undergraduate Research Symposium. p. 46. Shackouls Honors College, Mississippi State University. Apr. 13.
38. Durfey, C. L., **S. F. Liao**, D. Devost-Burnett, M. A. Crenshaw, C. S. Steadman, S. T. Willard, P. L. Ryan, H. Clemente, and J. M. Feugang. 2017. Assessment of growth and health performance of pigs born from magnetically nanopurified boar spermatozoa. *J. Anim. Sci.* 95 (Suppl. 2)/*J. Dairy Sci*. 100 (Suppl. 1): 40. Abstract was presented (oral and poster) by DCL the Annual Meeting of Midwestern Section/Branch of ASAS/ADSA, Omaha, NE. Mar. 13-15.
39. Wang, T., M. S. Hasan, M. A. Crenshaw, A. T. Sukumaran, T. Dinh, and **S. F. Liao**. 2017. Effect of dietary lysine on the skeletal muscle intramuscular fat content and fatty acid composition of late-stage finishing pigs. *J. Anim. Sci.* 95 (Suppl. 2)/*J. Dairy Sci*. 100 (Suppl. 1): 46-47. Abstract was presented (oral and poster) by MSH at the Annual Meeting of Midwestern Section/Branch of ASAS/ADSA, Omaha, NE. Mar. 13-15.

40. Hasan, M. S., T. Wang, S. Ching, J. M. Feugang, M. A. Crenshaw, L. S. Johnston, F. Chi, and **S. F. Liao**. 2017. Effect of a new montmorillonite-based feed additive on the production performance of newly weaned piglets. *J. Anim. Sci.* 95 (Suppl. 2)/*J. Dairy Sci*. 100 (Suppl. 1): 73-74. Abstract was presented (poster) by MSH the Annual Meeting of Midwestern Section/Branch of ASAS/ADSA, Omaha, NE. Mar. 13-15.
41. Moorhead, W. A., C. L. Durfey, **S. F. Liao**, D. Devost-Burnett, G. D. A. Gastal, P. L. Ryan, S. T. Willard, and J. M. Feugang. 2017. Effects of nanopurified boar semen for artificial insemination on protein detection in swine offspring muscle and fat tissue. *Reproduction, Fertility and Development*. 29(1): 139-139. Abstract was presentation (poster) by WAM at the 43rd IETS Annual Meeting, Austin, TX. Jan. 14-17.
42. Durfey, C. L., **S. F. Liao**, D. Devost-Burnett, T. Dinh, M. Crenshaw, S. T. Willard, P. L. Ryan, H. Clemente, and J. M. Feugang. 2017. Growth and market quality of pigs born from magnetic nanoparticle treated boar spermatozoa. Abstract (#69016). Poster presentation by CLD at the 43rd IETS Annual Meeting, Austin, TX. Jan. 14-17.

Theses:

1. Jones, A.K. 2017. The effect of poor maternal nutrition on offspring growth and maternal and offspring inflammatory status during gestation. PhD Diss. Univ. Connecticut, Storrs, CT.
2. Lisa C. Armbruster MS- Thesis August 2017: "Myogenic and Anabolic Gene Expression in Red and White Muscle from Sablefish (*Anaplopoma fimbria*) during Grow out".
3. Breann N. Sandberg MS- Thesis May 2017: " An Examination of the Effects of Dietary Rumen Protected- Histidine Supplementation on Finishing Beef Cattle Growth, Carcass and Meat Quality Parameters.
4. Elizabeth Hogan. The effects of virus-induced maternal inflammation on methylation patterns in skeletal muscle. MS Thesis December 2016.
5. Kellie Kroscher. Creation and characterization of mice with a mutation disrupting binding of a transcriptional repressor of insulin-like growth factor 2. MS Thesis August 2017

Other Publications and Presentations:

1. Selsby JT. The effect of heat stresses on porcine skeletal muscle. Project Director's meeting. Baltimore, MD, 7/13/17.
2. Turning down the heat: How heat stress affects muscle growth and limits pork production. Iowa Swine Day, Ames, IA, 6/29/17.
3. Spaulding H, Kelly EM, Sheffield JB, Quindry JC, Hudson MB, and Selsby JT. Impaired autophagic flux in dystrophic muscle augments extracellular autophagosome release. Advances in Skeletal Muscle Biology in Health and Disease. Gainesville, FL, March 8-10, 2017.
   *Note: Talk was awarded based on abstract (1/16 selected from ~120 submitted)
4. Baumgard L., SK Kvidera, EA Horst, MJ Dickson, JA Ydstie, CS Shouse, EJ Mayorga, M Al-Qaisi, S Lei, KL Bidne, JT Seibert, BJ Hall, AF Keating, JW Ross, **JT Selsby** and RP Rhoads. Consequences of leaky gut on the immune system, metabolism, physiology and animal performance. American Dairy Science Association. 2017.
5. Spaulding HR and **Selsby JT**, Autophagic dysfunction in dystrophic muscle is independent of disease progression. Iowa Physiological Society, Des Moines, IA, October 29th, 2016.
6. The heat is on: Heat stress-mediated changes in skeletal muscle. Modern Views of Nutrition Seminar Series. Iowa State University, 9/20/17.

7. It's getting hot in here: Heat stress-mediated changes in skeletal muscle. TriBeta Seminar 4/4/17.
8. **Selsby, JT**. Heat stress has effect on muscle growth, limits pork production. Feedstuffs. September 5th, p26-27, p32, 2017.
9. Boosting Heat Stress Tolerance in Turkeys. In Retaking the Field, Vol. 2, March 2017. A publication of the Supporters of Agricultural Research (SoAR) Foundation. Available at: http://supportagresearch.org/michigan-state-university-boosting-heat-stress-tolerance-in-turkeys/ Accessed December 7, 2017.
10. Invited Speaker at Texas Tech University. K.J. Thornton. Heat shock proteins and their role in meat quality. March 2017.