**Multistate Research Activity Accomplishments Report**

**Project/Activity Number**: NC1184

**Project/Activity Title**: Molecular Mechanisms Regulating Skeletal Muscle Growth and Differentiation

**Period Covered**: 10/01/2020-9/30/2021

**Date of This Report**: 11/28/21

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**Brief Summary of Minutes of Annual Meeting:**

The annual NC1184 technical committee meeting was held in mixed mode both in-person and virtually on October 28 and 29, 2021. The meeting was hosted by Dr. John Gonzalez of the Department of Animal Science, University of Georgia. On October 28th, the group was welcomed by Dr. Gonzalez, who shared information about the area, college, and department. The group then began with oral station reports.

On October 28th at 2 PM, the group had a conference call with Drs. Mark Mirando and Steven Smith, USDA/NIFA, who outlined current funding opportunities, the USDA NIFA budget, statistics on the number of proposals submitted annually and funding rates, and an update on NIFA’s move from Washington, D.C. to Kansas City. A question and answer session followed Drs. Mirando and Smith’s update. Dr. Sally Johnson updated the special issues of JAS. After the call with Drs. Mirando, Smith and Johnson, the groups continued with station reports.

The group voted to hold the 2023 meeting at the Washington State University, to be hosted by Dr. Min Du.

The 2022 meeting will be held at the University of Wisconsin-Madison, to be hosted by Dr. Wei Guo.

**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. Effect of maternal and post-hatch dietary inclusion of 25-hydroxycholecalciferal (25OHD3) on broiler chicken muscle growth characteristics and *in vivo* satellite cell activity
2. Determining the effects of dietary inclusion of the vitamin D metabolite, 25-hydroxycholecalciferol in commercial broiler production systems will positively impact the way the poultry industry feeds broiler breeder hens and their offspring to improve feed efficiency and meat yield.

**Connecticut Station:**

1. PCR arrays testing the effects of maternal diet on inflammatory factors have been completed, demonstrating sex by treatment and treatment effects on gene expression, including GATA3 and Toll-like receptor 1.
2. LPS challenge completed in 6-month-old offspring of over-, restricted-, and control fed ewes. Cytokine assays in progress as of fall 2021.
3. LD samples from 10-month-old offspring of over-, restricted- and control-fed rams sent to JMG at UGA for shear force testing.
4. Heart samples sent to W. Guo at UW Madison for analysis of autophagy associated pathways.

**Wisconsin Station:**

1. In collaboration with Dr. Reed (Connecticut Station), we found that overfeeding during gestation impacts autophagy signaling at late gestation and neonatal after birth in heart muscle. Cortisol level had no change at early and late gestation stages as well as neonatal. Overfeeding during gestation had significant interactions of maternal diet by developmental stages in male and female sheep. This work led to a manuscript that is under revision.

**Illinois Station:**

1. To determine the effect of maternal inflammation on offspring muscle and immune system development, pregnant sows were inoculated with lipopolysaccharide during mid-gestation. Work is ongoing to determine offspring muscle development, immune system programming, and lifetime productivity.
2. To determine the effects of myostatin on pig muscle growth, gene-editing was used to mutate myostatin in commercial pigs. To date, 77 pigs lacking functional myostatin have been produced but only 2 have survived beyond weaning. These animals do not display an increase in growth. Instead, they appear to be stunted in growth. Work is ongoing to determine the mechanism by which the loss of myostatin has different effects in pigs than in other species.

**Iowa Station:**

1. In collaboration with Dr. Rhoads (Virginia Tech) and Dr. White (TAMU), we are working to better understand metabolic dysregulation and muscle injury caused by heat stress. We are currently exploring the role of a mitochondrially target antioxidant as a countermeasure to heat stress-mediated dysfunction.
2. In conjunction with Dr. Rhoads (Virginia Tech) and Dr. White (TAMU), we have made further inroads into the role of biological sex on the muscular response to heat stress. We have completed an in vivo heat stress experiment and are working through analysis of phenotypical outcomes and will soon begin our biochemical and histological measures. We have also expanded our complement of tissues to include heart, which may provide novel insight as it is metabolically more oxidative than is oxidative skeletal muscle. Further, this may provide an opportunity to contribute to human health. Finally, we have recently generated some interesting data regarding whole-body metabolism/injury during the recovery from heat stress that suggests persistent changes as well as differences impacted by biological sex.
3. We initiated a new collaboration with an expert in proteomics technologies to more holistically capture changes to the proteome caused by muscle injury.

**Kansas Station:**

1. Overall energy production (mitochondrial respiratory chain and ATP synthase complexes) are up regulated during developmental muscle atrophy

**South Dakota Station:**

1. Evaluated the influence of feeding ractopamine HCl (400 mg/daily) to finsihing Holstein steers (Hergenreder et al., 2021b).
2. Evaluated the influence of recombinant bovine somatotropin on growth performance, skeletal muscle biological activity, and beta-adrenergic receptor expression in finishing heifers (Hergenreder et al., 2021a).
3. Evaluated the use of coated steroidal combination implants on feedlot performance and carcass traits of beef heifers fed for constant or varying days on feed (Ohnoutka et al., 2021).
4. Evaluated the effects of laidlomycin propionate and bacitracin zinc on digestibility coefficients and ruminal fermentation parameters using a continuous culture model (Thompson et al., 2021).
5. A review over the effects of dust on feedlot cattle was also conducted (Urso et al., 2021).
6. Evaluated the influence of vitamin A status in finishing steers on myogenic gene expression and skeletal muscle fiber characteristics (Wellmann et al., 2021).
7. Investigated the influence that cane molasses supplementation has on gene expression and protein abundance of ruminal epithelial tissue in transition dairy-cow diets (Miller et al., 2021).
8. Finally, a study to determine the effects that encapsulated methionine fed during the growing period has on skeletal muscle growth and finishing phase performance and carcass characteristics (Baggerman et al., 2021).

**Utah Station:**

1. Demonstrated that implanting cattle with anabolic hormones impacts liver mineral concentrations.
2. Improved understanding of how cattle of different breed types respond to different anabolic implant procedures.
3. Learned about the pathways through which anabolic hormones (estradiol and trenbolone acetate) improve skeletal muscle growth in both the *longissimus lumborum* of beef steers and primary bovine satellite cells.

**Virginia Station:**

1. O-GlcNAcylation is a post-translational modification considered to be a nutrient sensor that reports nutrient scarcity or surplus. Although O-GlcNAcylation exists in a wide range of cells and/or tissues, its functional role in muscle satellite cells (SCs) remains largely unknown. Using a genetic approach, we ablated O-GlcNAc transferase (OGT), and thus O-GlcNAcylation, in SCs. We first evaluated SC function *in vivo* using a muscle injury model and found that OGT deficient SCs had compromised capacity to repair muscle after an acute injury compared to the wild-type SCs. We generated muscle specific OGT and interleukin-15 receptor alpha subunit (IL-15rα) double knockout mice (mDKO). Deletion of IL-15rα in skeletal muscle impaired IL-15 secretion. When fed a high-fat diet, mDKO mice were no longer protected against HFD-induced obesity compared to wild-type mice.
2. OGT leads to a lean phenotype through enhanced interleukin-15 (IL-15) expression though only an association.
3. Postnatal muscle growth is accompanied by increases in fast fiber type compositions raising the possibility that a slow to fast transition may be partially requisite for increases in muscle mass. We ablated the mouse *Myh4* gene (myosin heavy chain IIB) and examined its consequence on postnatal muscle growth using chemical and genetic modifiers of muscle fiber type composition.
4. Pork quality is a product of the rate and extent of muscle pH decline paced by carbohydrate metabolism *postmortem*. Beta-adrenergic agonist ractopamine alters muscle metabolism but has little impact on pork quality. We fed pigs over a four week period and determined glycolytic metabolites during the postmortem period.
5. Chronic heat stress causes several metabolic adaptations, which limit animal growth and performance. Our lab has demonstrated that HS has negative impacts on substrate metabolism by reducing fat oxidation and metabolic flexibility, which reduces overall ATP production. Data collected from chronic HS studies (3-weeks) demonstrates that pigs with greater metabolic flexibility at the conclusion of HS experienced lower core temperatures throughout the event.

**Indiana Station:**

1. Activities: Used cell and molecular biology techniques, animal models and production animals to study molecular regulation of muscle growth and metabolism.
2. Short-term outcomes: Trained 3 postdoctoral fellows (two landed faculty jobs), currently training 11 graduate students and 6 undergraduate students in various research projects; developed new research techniques and methods; used state-of-art single cell RNA sequencing to illustrate cell dynamics and reveal novel subpopulations in murine and porcine skeletal muscles.
3. Outputs: Presented 4 seminars to the general publics and research communities; Published 11 peer-reviewed papers; Carried out 4 funded projects.

**Texas Station:**

1. Found conjugated linoleic acid supplementation increased skeletal muscle mitochondrial function in young horses while decreasing antioxidant enzyme activity with no change in oxidative stress. Therefore, CLA may be decreasing oxidative load in skeletal muscle, minimizing the need for traditional antioxidant enzyme pathways (superoxide dismutase/glutathione peroxidase).
2. Found that complexed trace mineral supplementation to young, exercising horses increased skeletal muscle antioxidant activity, both at rest and following a trailering stressor.
3. Found that supplementation of a postbiotic modulated cellular stress and inflammation favorably following a submaximal exercise stressor in young horses.
4. Found impaired mitochondrial function but greater mitochondrial capacities, greater type IIx fibers, lesser type IIa/x hybrid fibers, and greater satellite cells/muscle fiber in aged compared to young horses. Submaximal exercise training improved skeletal muscle mitochondrial capacities in both age groups of horses.
5. Found increased mitochondrial function in Brahman calves that had been stressed in utero. May have implications for future growth potential and efficiency of production.
6. Continue identification of impacts of temperament and breed on skeletal muscle mitochondrial energetics and relationships to meat product quality in beef cattle.

**Arkansas Station:**

1. DHA is a potent adipogenic and lipogenic factor that can change the metabolic profile of muscle cells by increasing myocellular fat.
2. Higher dietary crude protein (CP) levels promote growth performance for finishing lambs, whereas lower dietary CP levels are beneficial for meat quality, especially when evaluating color characteristics in the final product.
3. Black pigs (BP) have superior intramuscular fat content to cross-bred commercial pigs (CP), while the growth performance of CP was better, and the transcriptomic differences between these two groups of pigs may cause the meat quality and growth performance variance
4. Heat stress and feed restriction distinctly affect performance, carcass and meat yield, intestinal integrity, and inflammatory (chemo)cytokines in broiler chickens.
5. Neuropeptide Y and its receptors are expressed in chicken skeletal muscle and regulate mitochondrial function.
6. Phytogenic feed additives improve broiler feed efficiency via modulation of intermediary lipid and protein metabolism-regulated signaling pathways.

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

**Alabama Station:**

1. Ongoing work is being conducted regarding the in vitro differentiation capacity of muscle satellite cells from affected and unaffected broilers
2. Effect of early and late-stage incubation temperature variation on broiler chicken muscle satellite cell activity and incidence and severity of Wooden Breast myopathy at processing

**Georgia Station:**

1. *In ovo* feeding of high-yield broiler embryos with 2.5 mM of NR increased pectoralis major weight and length by 12 and 7%, respectively.
2. Increased pectoralis major morphometrics was accompanied by a 21% decreased in muscle fiber cross-sectional area and 35% increase in muscle fiber density.
3. Fiber morphometric results were associated with increased mRNA expression of SIRT-1, Cyclin D1, D2, and D3 at embryonic day 15, 18, and hatching.

**Kansas Station:**

1. Human pathogenic alleles of TRIM32 expressed in *Drosophila* muscle tissue result in progressive muscle degeneration.
2. The protein kinase NUAK (mammalian ARK5) coordinates with the insulin signaling pathway to control organismal size and sarcomere remodeling.

**Wisconsin Station:**

1. RNA splicing factor RBM20 is differentially expressed in different muscle types and regulates myogenesis and muscle hypertrophy in a muscle type dependent manner.

**Utah Station:**

1. Gained insight into how ultrasonication impacts mitochondrial function leading to development of tenderness in beef animals.
2. Developed a model to study dark cutting beef in cattle

**Virginia Station:**

1. IR, IRS-1, PDK1, mTORC2, pan-Akt, Akt1, and Akt2, play an important role in the activation of translation initiation in response to the insulin surge after an intermittent bolus meal.
2. We also identified the amino acid sensors Sestrin1 and 2, and Rag A/C and Rheb as being sensitive to amino acid pulse after a meal.
3. Our work also suggest that skeletal muscle protein synthesis is differentially regulated compared to other organs in the body.

**Hawaii Station:**

1. Hawaii station continued to produce recombinant proteins suppressing the bioactivity of myostatin (MSTN), a negative regulator of muscle growth.
2. MSTN propeptide (MSTNpro) is a strong negative regulator of MSTN, thus a study was developed to produce recombinant mouse MSTN fragment containing amino acid sequences 1-218 (mMSTNpro218) fused to mouse IgG Fc domain (mMSTNpro218-mFc).
3. mMSTNpro218-mFc cDNA was synthesized and ligated into the pcDNA-3.1+ plasmid (mMSTNpro218-mFc-pcDNA-3.1) commercially. A large scale of mMSTNpro218-mFc-pcDNA-3.1 plasmid was produced from transformed *E. coli* cells.
4. The Expi293™ Expression System (ThermoFisher Scientific, MA, USA) was used to express mMSTNpro218-mFc proteins following the protocol described by the manufacture.
5. The expression of the mMSTNpro218-mFc protein was confirmed by SDS-PAGE and Western blot analyses.
6. mMSTNpro218-mFc was purified by Protein A affinity chromatography but a large portion of mMSTNpro218-mFc in the cell culture did not bind to Protein A. SDS-PAGE in non-reduced condition showed that the protein aggregated in non-reduced condition, suggesting that the low affinity of the protein to the protein A column was probably due to the aggregation.
7. When bioactivity of the mMSTNpro218-mFc was measured using the (CAGA)12-luciferase reporter gene assay, the MSTN-inhibitory capacity of the protein was extremely low. The aggregation of mMSTNpro218-mFc is likely associated with the low MSTN-inhibitory potency of recombinant mMSTNpro218-mFc. Thus, future studies need to find a way to minimize the aggregation of the expressed protein.

**Washington Station:**

1. The Washington State Station is continuing to define the impacts of maternal nutrition on early skeletal muscle development, and found that obesity during the early development attenuates myogenic differentiation.
2. The Washington State Station continues to define the molecular mechanisms regulating early formation of muscle cells and test the role of retinoic acid signaling in embryonic development.

**Arkansas Station:**

* + - 1. Hypoxia further exacerbates woody breast myopathy in broilers via alteration of satellite cell fate.

**Ohio Station:**

Effect of Thermal Stress on Pectoralis Major Satellite Cells (with Michigan Station)

1. Poultry selected for growth have an inefficient thermoregulatory system and are more sensitive to temperature extremes
2. Cold temperatures inhibited rates of proliferation and differentiation of p. major muscle satellite cells.
3. If the hot temperature was applied during p. major muscle satellite cell proliferation, it resulted in greater stimulatory effects on differentiation than if the hot temperature was administered only during differentiation. Similarly, cold temperature administered during proliferation tended to have more suppressive effects on differentiation than if the cold temperature was applied only during differentiation.
4. Growth selection has resulted in the p. major muscle satellite cells from the faster-growing commercial turkeys to be more sensitive to hot temperature during both proliferation and differentiation. The increased rates of proliferation and differentiation in vivo may result in a greater potential to accrete more satellite cells to drive myofiber hypertrophy, which could impact post-hatch breast muscle growth and structure.
5. Adipogenic gene expression is more responsive to thermal challenge in proliferating satellite cells than in differentiating satellite cells, and that growth-selection has increased temperature sensitivity of satellite cells.

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

**California Station**

1. Tracked white striping, gross muscle pathology, and histological evidence of muscle

pathology. With these findings we have developed linear regression models that show a

positive correlation between white striping, gross pathology, and histopathology relative to weight and age.

**Kansas Station:**

1. The E3 ubiquitin ligase TRIM32 is required to maintain protein levels of the costamere components βPS integrin and Sarcoglycan δ.
2. Proteasome and peptidase proteins are selectively upregulated during developmental muscle atrophy. Thin filament-associated proteins are preferentially degraded before thick filament proteins.

**Virginia Station:**

1. We set out to determine how the frequency of nutrient delivery controls protein deposition in neonatal muscles. Our studies indicated that despite receiving the same amount of diet per unit of body weight, the efficiency of protein deposition was greater in pigs fed intermittently compared to those fed continuously. This increase is ascribed to a greater stimulation of protein synthetic pathways by intermittent feeding and occurred in response to a rise in insulin and amino acids. However, the relative contributions of the insulin- and amino acid-signaling pathways that mediate the effects of these different feeding modalities on protein synthesis and degradation have not been fully elucidated.

**New Jersey Station:**

1. The NJ Station is developing the necessary methodology to study the transcriptome and translatome in skeletal muscle. Development of RNA sequencing and ribosomal profile in mouse tissues as well as measurement of protein synthesis using deuterium oxide will provide new technical platforms to investigate skeletal muscle protein homeostasis in response to nutrients and activity.

**Texas Station:**

1. Found that susceptibility to occurrence of dark cutting carcasses is genetically influenced by sire. This finding will aid selection efforts for reducing incidence.
2. Identified specific microRNAs expressed at harvest that are associated with dark cutting incidence – a first step in understanding genetic triggers that participate in the stress-associated physiological mechanisms that result in dark cutting carcasses.

**Impact Statements:**

Objective 1:

Changes in knowledge:

1. Information regarding the impacts of various nutrients in production animal species on muscle development and growth characteristics will ultimately improve the efficiency of meat production and sustainability of commercial livestock industries in the USA.
2. Maternal diet impacts offspring basal inflammatory profile in a sex and diet dependent manner.
3. Offspring of over-fed ewes had increased tenderness relative to offspring of control- and restricted-fed ewes.
4. Over-fed ewes during gestation affected autophagy signaling at late development stage.
5. Dysregulation of autophagy is emerging as a common cellular dysfunction in a variety of pathological conditions including muscular dystrophy and heat stress. Stimulation of autophagy may provide immediate and profound impacts to the agricultural and biomedical communities.
6. Heat stress continues to negatively impact swine production and will do so at progressively greater magnitudes should environmental models be accurate and/or pigs continue to be selected for protein accretion. Our discovery of a sex effect of heat stress could lead to renewed focus of sexed semen, influence the location of new barn construction, and barrow and gilt management strategies.
7. Evaluated technologies that influence finishing cattle growth production in the U.S. and published our findings in high impact journals for the animal science discipline.
8. O-GlcNAcylation plays a critical role in the maintenance of SC health and function in normal and injured skeletal muscle.
9. Double knockout mice suggest OGT action is partially mediated by muscle IL-15 production. Our work suggests that interfering the OGT-IL15 nutrient sensing axis may provide a new avenue to combating obesity and its related metabolic disorders and provide some clarity into how the O-GlcNAc nutrient signaling pathway leads to a lean phenotype.
10. Expression of type IIB myosin heavy chain is necessary for postnatal fiber number maintenance and hypertrophy in growing mice.
11. RAC feeding reduces glycogen content and diminishes lactate accumulation postmortem, but raises questions about the role glycolytic flux has in driving pork quality development.

Objective 2:

Changes in knowledge:

1. Information regarding the role myogenic stem cells (satellite cells) may play in the development of the costly myopathy known as Wooden Breast currently impacting the commercial broiler chicken industry was presented at key poultry industry stakeholder meetings. This information adds to the body of knowledge surrounding the Wooden Breast condition and will aid the poultry industry in furthering the investigation into the cause and strategies to eliminate this costly meat quality defect from the poultry meat supply.
2. Low-dose *in ovo* nicotinamide riboside feeding alters the muscle fiber profile in a manner that may affect post-hatch growth and meat quality
3. We were able to demonstrate that IUGR skeletal muscle and myoblast deficits were associated with adaptive changes in β adrenergic based on their responsiveness to clenbuterol. These mechanisms represent potential therapeutic targets for improving growth and metabolic outcomes in IUGR-born offspring.
4. Dysregulation of autophagy is emerging as a common cellular dysfunction in a variety of pathological conditions including muscular dystrophy and heat stress. Stimulation of autophagy may provide immediate and profound impacts to the agricultural and biomedical communities.
5. Developed a fundamental understanding of the cell’s intrinsic molecules and extrinsic signals that regulate skeletal muscle development, growth and regeneration.
6. Research related to this project will lead to fundamental understanding of the cell intrinsic molecules and extrinsic signals that regulate skeletal muscle development, growth and regeneration. Such knowledge will serve as the foundation for translational approaches to increase meat production and improve meat quality in animal agriculture, and to improve health of the muscular system of humans.
7. Understanding the roles of retinoic acid, a key metabolite of vitamin A, in fetal and neonatal muscle development and its long-term impacts on beef production and quality will fill an important knowledge gap.
8. Our discoveries emphasize the importance of maternal nutritional management in improving fetal development and the long-term performance of animals.
9. Thermal stress may have long-term implication on breast meat quality through changes in muscle development and growth potential.
10. Growth selection plays a role in the response of the breast muscle to thermal stress
11. Immediate posthatch thermal stress both cold and hot effects both the proliferation and differentiation of satellite cells required for muscle growth and muscle mass accretion.

Objective 3:

1. The New Jersey Station developed the necessary methodology to study the transcriptome and translatome in skeletal muscle. Development of RNA sequencing and Ribo-seq in mouse tissues as well as measurement of protein synthesis using deuterium oxide will provide new technical platforms to investigate skeletal muscle protein homeostasis in response to nutrients and activity in collaboration with other NC1184 investigators.

**Collaborative Grants:**

Selsby, Baumgard, Rhoads, and White. USDA/AFRI Foundation Grant, “Calcium regulation as a contributor to heat stress-mediated skeletal muscle dysfunction,” $500,000, 1/1/21-12/31/23.

Selsby, Baumgard, Ross, Schulz, Ramirez, Serao, Rhoads, and White, USDA/AFRI IDEA Grant, “Basic and applied consequences of heat stress in barrows and gilts,” $1,000,000, 9/1/20-8/30/25.

Selsby, Baumgard, and Rhoads. USDA/AFRI Foundation Grant, Administrative supplement (2017-05931), $37,241, 1/1/18-12/31/21.

Selsby, Baumgard, and Rhoads. USDA/AFRI Foundation Grant, Therapeutic approaches to heat stress: targeting mitochondria (2017-05931), $497,000, 1/1/18-12/31/21.

Myokines: an avenue for improved growth; PD – G.K. Murodoch; Co-PD – K.J. Thornton (30%), B.M. Murdoch, G. Chibisa; USDA Grant #2020-02690; $200,000 awarded; 01/21-12/22

NSF Engineering Research Center proposal: Cellular Agriculture in Food Engineering and Manufacturing, University of Nebraska, Purdue University and University of Connecticut. Co-I in Thrust 1 (Cells and Bioinks).

Strasburg, G., Velleman, S.G., Reed, K. NIFA AFRI, “Turkey Breast Muscle Development: The Biological Response to Thermal Challenge in Production Birds,” $500,000, 4/1/2020-3/31/23.

**Collaborative Publications:**

Moyorga EJ, Horst EA, Goetz B, Abeyta M, Al-Qaisi MA, Lei S, **Rhoads R, Selsby JT**, and Baumgard LH. Rapamycin administration during an acute heat stress challenge in growing pigs. Journal of Animal Science. In Press, 2021.

Rudolph TE, Mayorga EJ, **Rhoads RP**, Baumgard L, and **Selsby JT**. The effect of MitoQ on heat stressed skeletal muscle, and the potential confounding effect of biological sex. Journal of Thermal Biology. In Press. 2021

Fausnacht DW, Kroscher KA, McMillan R, Davy DP, Baumgard LH, **Selsby JT**, Hulver MW, and **Rhoads RP**. Heat Stress Reduces Metabolic Rate While Increasing Respiratory Exchange Ratio in Swine. Animals. In Press. 2021

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Baggerman, J. O., A. J. Thompson, M. A. Jennings, J. E. Hergenreder, W. Rounds, Z. K. Smith, and B. J. Johnson. 2021. Effects of Encapsulated Methionine on Skeletal Muscle Growth and Development and Subsequent Feedlot Performance and Carcass Characteristics in Beef Steers. Animals 11(6):1627.

Hergenreder, J. E., J. O. Baggerman, T. L. Harris, A. J. Thompson, K. S. Spivey, P. R. Broadway, G. J. Vogel, Z. K. Smith, and B. J. Johnson. 2021a. Bovine Somatotropin Alters Myosin Heavy Chains and Beta Receptors in Skeletal Muscle of Feedlot Heifers with Little Impact on Live or Carcass Performance. Meat and Muscle Biology 5(1):1-16. doi: <https://doi.org/10.22175/mmb.11137>

Hergenreder, J. E., J. L. Beckett, Z. K. Smith, and B. J. Johnson. 2021b. A Greater dose of Ractopamine Hydrochloride Enhances Feedlot Performance and Impacts Carcass Characteristics of Calf-Fed Holstein Steers. Am J Anim Vet Sci 16(1)doi: 10.3844/ajavsp.2021.99.104

Miller, W. F., E. C. Titgemeyer, T. G. Nagaraja, D. H. M. Watanabe, L. D. Felizari, D. D. Millen, Z. K. Smith, and B. J. Johnson. 2021. Influence of Cane Molasses Inclusion to Dairy Cow Diets during the Transition Period on Rumen Epithelial Development. Animals 11(5):1230.

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Thompson, A. J., Z. K. Smith, J. O. Sarturi, and B. J. Johnson. 2021. Antimicrobial supplementation alters digestibility and ruminal fermentation in a continuous culture model. J Appl Anim Res 49(1):23-29. doi: 10.1080/09712119.2021.1876704

Urso, P., A. Turgeon, F. Ribeiro, Z. Smith, and B. Johnson. 2021. Review: The Effects of Dust on Feedlot Health and Production of Beef Cattle. J Appl Anim Res doi: 10.1080/09712119.2021.1903476

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1. **Morgan Zumbaugh,** December, 2020.

**Other Publications and Presentations:**

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