Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

General Highlights

- NCCC210 members and *ad hoc* attendees: Werner Bergen, Brynn Voy, Min Du, Stone Ding, Kola Ajuwon, Ching Hu, Woo Kyun Kim, Theo Van Kempen, Kimberly Barnes, Brian Piccolo, Laxmi Yeruva, and several postdocs/students
- The meeting was held at the Coach House at the Glessner House Museum following the tradition of hosting the meeting at a non-EB sanctioned venue over the past few years. Glessner Museum had signs posted on the doors and provided A/V. D'Absolute Catering provided muffings, bagels, coffee, and lunch.
- The meeting began promptly at 9am and Dr. Bergen provided a brief synopsis of the history of the meetings.
- The group discussed the completed renewal objectives and then confirmed last year's decision to continue meeting before EB even though ASN will no longer be affiliated with EB meetings.
- Discussion then centered around inviting colleagues and younger faculty members to ensure future meetings
- Two Co-Chairs, Kola Ajuwon and Kim Barnes, were elected for 2018 in anticipation of the next renewal cycle in 2019. Woo Kyun Kim will serve as Chair for the 2018 meeting.
- Research updates were given by the majority of participants from 10:10am to 4pm.
- After the conclusion of the official meeting, a guided tour of the Glessner House was made available for interested members.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 1

Auburn University (Werner Bergen, Terry Brandebourg)

The Bergen and Brandebourg growth biology laboratories in Animal Science at Auburn University have overlapping goals for their activities. The labs may pursue collaborative work or distinctly different projects. The Bergen lab has participated in collaborative studies emphasizing plant omega 3 fatty acid sources on swine production. We have completed studies on meat quality, animal performance, fatty acid analyses and gene expression in muscle and adipose tissues. The first publication from this series of studies is listed below The lab continues to collaborate in work on by-product feeding on lipid gene expressions in grower and finishing pigs.

The Brandebourg Lab continues to focus on molecular regulation of residual feed intake, molecular regulation of marbling in cattle, and the development of a swine model that will produce obesity without feeding high fat diets.

Accomplishments

Bergen involved in Objectives 1,2,4. Papers where published including a review of 50 years of meat animal growth research with contemporary focus on adipose and muscle stem cells.

Work in the Brandebourg lab directly addresses Objectives 2 and 4 and could also enhance Objective 5, especially with the establishment of the Mangalica pig as a novel model of hyperphagic obesity that spontaneously develops indices of metabolic syndrome.

Publications:

Campos CF, Duarte MS, Guimarães SE, Verardo LL, Wei S, Du M, Jiang Z, Bergen WG, Hausman GJ, Fernyhough-Culver M, Albrecht E, Dodson MV.
Review: Animal model and the current understanding of molecule dynamics of adipogenesis.
Animal. 2016 Jan 18:1-6.

Bergen W.G. and Brandebourg T.D. 2016. Parallels between agriculture and human physiology Regulation of lipid deposition in farm animals:. Exp Biol Med (Maywood). 2016 Jun;241(12):1272-80. doi: 10.1177/1535370216654996.

Adhikari, CK, LI Chiba, SD Brotzge, MS Vieira, C Huang, WG Bergen, CL Bratcher, S Rodning, and EG Welles 2017. Early dietary amino acid restrictions and flaxseed oil supplementation on the leanness of pigs and quality of pork: Growth performance, serum metabolites, carcass characteristics, and physical and sensory characteristics of pork. Livestock Science No. LIVSCI-D-16-986R1. Accepted

Impact

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

The role of NCCC210 (and its predecessors) in understanding of animal lipid metabolism has been enormous. This Multistate committee has been at the forefront in lipid metabolism topics germane to animal production and development of muscle–foods products consistent with the nutritional needs of the consuming public since the 1940s. At Auburn, new-global laboratory techniques and bioinformatics have been utilized to delineate molecular regulation of marbling and also feed efficiency. Initial rounds of these studies (as elsewhere) will require extensive additional work to develop paradigms which consistently will result in high production efficiency and "healthy" animal foods products by the US livestock industry.

Of particular interest are data from the Brandebourg laboratory indicating the Mangalica pig exhibits superior meat quality and Red Mangalica could serve as a unique model for studying marbling. Also as Mangalica pigs become fatter, they spontaneously develop risk factors associated with metabolic syndrome: obesity, hyperglycemia, hyperinsulinemia, dyslipidemia, and low-grade chronic inflammation. Thus, Mangalica pigs now represents a novel pig model that develops metabolic syndrome simply by being allowed to eat to their voluntary intake. Also, these pigs represent a novel model to study feed intake as they eat 2-3 times more than is necessary to maximize bone and muscle growth unlike other swine breeds that essentially "eat to their energy". Furthermore, feeding high fat diets fails to suppress voluntary feed intake in these pigs indicating they are a novel model of hyperphagia. Thus we now have the necessary data to satisfy review panels and study sections for both NIH and USDA funding mechanisms which can now be targeted.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 2

Washington State University (Min Du)

Accomplishments:

The Nutrigenomics and Growth Biology laboratory at Washington State University continues to define epigenetic mechanisms regulating early adipogenic commitment and differentiation, especially brown/beige adipogenesis. His laboratory found that AMP-activated protein kinase is critical in the early stage of brown/beige adipogenesis via enhancing the production of alpha-ketoglutarate. In addition, his group also found that polyphenolic compounds such as resveratrol promote beige adipogenesis, which prevents metabolic dysfunction in mice challenged by high fat diet.

Publications:

1. Wang B., X. Fu, X. Liang, Z. Wang, Q. Yang, T. Zou, W. Nie, J. Zhao, P. Gao, M. J. Zhu, J. M. De Avila, J. Maricelli, B. D. Rodgers, and **M. Du**. (2017). Maternal retinoids increase PDGFRa progenitor population and beige adipogenesis in progeny by stimulating vascular development. *EBioMedicine*, In press.
2. Guan L., X. Hu, L. Liu, Y. Xing, Z. Zhou, X.W. Liang, Q. Yang, S. Jin, J. Bao, H. Gao, **M. Du**, J. Li, and L. Zhang. (2017). *Bta-miR-23a* involves in adipogenesis of progenitor cells derived from fetal bovine skeletal muscle. *Scientific Report*, In press.
3. Griner, J. D., C. J. Rogers, M. J. Zhu, and **M. Du.** (2016). Lysyl oxidase propeptide promotes adipogenesis through inhibition of FGF-2 signaling. *Adipocyte*, In press.
4. Zou, T., Q. Yang, B. Wang, M. Zhu, P. W. Nathanielsz, and **M. Du**. (2017). Resveratrol supplementation to high fat diet-fed pregnant mice promotes brown and beige adipocyte development and prevents obesity in male offspring. *Journal of Physiology*, 595: 1547-1562.
5. Wang, S., X. Liang, Q. Yang, X. Fu, M. Zhu, B.D. Rodgers, Q. Jiang, M. V. Dodson, and **M. Du**. (2017). Resveratrol enhances brown adipocyte formation and function by activating AMP-activated protein kinase (AMPK) α1 in mice fed high-fat diet. *Molecular Nutrition and Food Research*, In press.
6. Kang, Y., Xue, Y., **M. Du**, M. J. Zhu. (2016). Preventive effects of Goji berry on dextran sulfate sodium induced colitis in mice. *Journal of Nutritional Biochemistry*, 40: 70-76.
7. Liang, X. W., Q. Y. Yang, L. P. Zhang, J. Maricelli, B. D. Rodgers, M. J. Zhu, and **M. Du** (2016). Maternal high-fat diet during lactation impairs thermogenic function of brown adipose tissue in offspring mice. *Scientific Reports*, 6: 34345.
8. Yang, Q., X. Liang, X. Sun, L. Zhang, X. Fu, C. J. Rogers, A. Berim, S. Zhang, S. Wang, B. Wang, M. Foretz, B. Viollet, D. R. Gang, B. D. Rodgers, M. Zhu, and **M. Du**. (2016). AMPK/α-ketoglutarate axis dynamically mediates DNA demethylation in the Prdm16 promoter and brown adipogenesis. *Cell Metabolism*, 24: 542-554.
9. Wei, S. J., **M. Du**, Z. H. Jiang, G. J. Hausman, L. F. Zhang, and M. V. Dodson. (2016). Long noncoding RNAs in regulating adipogenesis: new RNAs shed lights on obesity. *Cellular and Molecular Life Sciences*, 73: 2079-2087.

10. Wang, B., Q. Yang, C. L. Harris, M. L. Nelson, J. R. Busboom, M. J. Zhu, and **M. Du**. (2016). Nutrigenomic regulation of adipose tissue development – role of retinoic acid. *Meat Science*, 120: 100-106.
11. Liang, X., Q. Yang, X. Fu, C. J. Rogers, B. Wang, H. Pan, M. J. Zhu, P. W. Nathanielsz, and **M. Du**. (2016). Maternal obesity epigenetically alters visceral fat progenitor cell properties in male offspring mice. *Journal of Physiology*, 594: 4453-4466.
12. Campos, C. F., M. S. Duarte, S. E. F. Guimaraes, L. L. Verardo, S. Wei, **M. Du**, Z. Jiang, W. G. Bergen, G. J. Hausman, M. Fernyhough-Culver, E. Albrecht, and M. V. Dodson. (2016). Review: Animal model and the current understanding of molecular dynamics of adipogenesis. *Animal*, 10: 927-932.
13. Li, N., Q. Y. Yang, R. Walker, T.B. Thompson, **M. Du**, and B. R. Rodgers. (2016). Myostatin attenuation in vivo reduces adiposity, but activates adipogenesis. *Endocrinology*, 157: 282-291.
14. Miao, Z. G., L. P. Zhang, X. Fu, Q. Y. Yang, M. J. Zhu, M. V. Dodson, and **M. Du**. (2016). Invited review: Mesenchymal progenitor cells in intramuscular connective tissue development. *Animal*, 10: 75-81.
15. Fu, X., M. J. Zhu, S. Zhang, F. Marc, V. Benoit, and **M. Du**. (2016). Obesity impairs skeletal muscle regeneration via inhibition of AMP-activated protein kinase. *Diabetes*. 65: 188-200.
16. Zhang, H., **M. Du**, Q. Yang, and M. J. Zhu. (2016). Butyrate suppresses murine mast cell proliferation and cytokine production through inhibiting histone deacetylase. *Journal of Nutritional Biochemistry*, 27: 299-306.

Impacts:

The worldwide epidemic of obesity poses a serious problem, which is closely associated with many chronic diseases, including cardiovascular diseases, type 2 diabetes, cancers and other diseases, which incur direct medical costs around $200 billion per year, plus human capital loss exceeding $500 billion in the USA alone! Since the recent discovery of beige adipocytes, the central role of brown and beige fat in prevention of obesity and metabolic dysfunction has been rapidly recognized. Knowledge obtained through these studies in brown/beige adipogenesis will eventually translate into clinical practice, reducing obesity and metabolic diseases. In addition, adipose tissue development also has profound impacts on animal production efficiency and meat quality. Thus, knowledge obtained also has important applications to animal production.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 3

West Virginia University (Kimberly Barnes)

Our lab has had the focus of the effect of dietary lipids on lipid metabolism and body composition, specifically focused on CLA and its interaction with the other lipids in the diet and sources of omega-3 fatty acids other than fish oil. Due to a change in focus for me and corresponding change to my faculty appointment to only 10% research no ongoing research is currently occurring in my lab but we did submit and present two abstracts in the past year.

Presented at the 2016 Joint Annual Meeting of ADSA and ASAS, July 2016, Salt Lake City, UT:

Relationship between antioxidants and residual feed intake in grazing heifers

J.N. Kidrick, E.E. Felton, K.S. Shaffer, and K.M. Barnes
Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV 26506

Residual feed intake (RFI) has been established to be a more accurate measure of feed efficiency; however, questions remain about the specific factors that contribute to individual variation. It has been determined that heat production from metabolic processes, body composition, and physical activity explain a large proportion of the variation but not 100%. Additionally, because RFI is an expensive trait to measure, identification of an easily measured biomarker of RFI would be beneficial in animal selection. Our current objective was to determine if antioxidant status contributes to variation in RFI. Serum was collected from a group of genetically similar heifers that were maintained on the same diet. During the feeding period, feed intake and body weight were recorded and used in the calculation of ADG and RFI. Serum nitric oxide (n = 48), glutathione peroxidase (n = 34), and total antioxidant capacity (n = 34) were measured using colorimetric assay kits. Nitric oxide levels were estimated using a nitrate/nitrite kit. Serum nitric oxide tended (P = 0.08) to be positively (r = 0.26) correlated with RFI. Glutathione peroxidase and total antioxidant capacity were not correlated with RFI, but total antioxidant capacity did tend (P = 0.06) to be negatively (r = -0.33) correlated with ADFI. Our data indicate that total antioxidant status may not be an adequate indicator of RFI. Increased intake may require a greater utilization of antioxidants, thus the reduced levels we observed in high intake heifers. The positive correlation we observed between RFI and nitric oxide could indicate less tissue turnover in the more efficient animals (low RFI). Therefore nitric oxide does show some potential as a biomarker of RFI.

To be presented at the 2017 Experimental Biology Meetings, April 22, 2017, Chicago, IL:

Effect of Dietary Coconut Oil and Conjugated Linoleic Acid on Liver Metabolic Phenotype in Mice

Kimberly M. Barnes, Jake P. Engle, Qiannan Chen, Anna M. DiGregorio, and Joseph W. McFadden
Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV 26506

Mice fed coconut oil (CO) lose more body fat when fed conjugated linoleic acid (CLA) than mice fed soy oil (SO), in part due to increased basal lipolysis. These mice also have increased liver fat content but

changes in specific liver metabolites are largely unknown. Our objective was to use mass spectrometry-based metabolomics to determine the metabolic profile of mice fed CO and/or CLA. Male mice from 4 common strains (CF-1, Swiss Webster, ICR, and NIH; n= 24 each) were obtained at weaning, fed either CO or SO for 6 wk, then 0 or 0.5% CLA for an additional 12 d. Following liver homogenization and methanol extraction, metabolites were profiled using electron impact ionization, gas chromatography, and mass spectrometry. Mass spectral analyses with standards resulted in a total of 37 identifiable metabolites. For untargeted metabolomics data, statistics were performed on the log of the normalized and autoscaled data. As reported previously, we detected an oil x CLA interaction ($P < 0.05$) on body fat, with CO+CLA-fed mice being the leanest. Basal lipolysis was increased by both CO ($P < 0.001$) and CLA ($P < 0.01$), with CO+CLA-fed mice having the greatest rate of NEFA release. Principle component analysis (PCA) revealed that the first three components accounted for 36% of the variation in the samples, and no more than three metabolites fell outside the 95% confidence ellipse. For the first three components, metabolites with variable importance of projection (VIP) scores greater than 2.0 were linoleic acid and oleic acid. PCA also indicated that dietary oil (CO or SO) had a greater effect on liver metabolites than CLA. CO-fed mice also showed less variation in metabolites when fed CLA than did SO-fed mice. Eleven metabolites were altered by diet, with fatty acid changes that correspond with the dietary oils being the most significant. Other metabolites altered include phosphoric acid (decreased by CO, $P < 0.01$), proline (decreased in CO+CLA-fed mice vs all other diets, $P < 0.05$), β-hydroxybutyrate (BHBA; increased in CO+CLA-fed mice vs SO and SO+CLA-fed mice with CO-fed mice being intermediate, $P < 0.05$), and acetic acid and isoleucine (both decreased in CO+CLA-fed mice vs SO and SO+CLA-fed mice, $P < 0.05$). Very few differences were detected between the different strains of mice. The increased lipid mobilization in the CO+CLA-fed mice may account for the increased ketone bodies and require greater protein synthesis as well, thus accounting for the decreased amino acid concentrations. This is supported as BHBA was negatively correlated with linoleic acid, valine, and phosphoric acid ($P < 0.05$; $r = -0.34$ to $-0..24$) and positively correlated with glycerol ($P < 0.05$; $r = 0.25$).

No publications in 2016.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 4

University of Georgia (Woo Kyun Kim)

1. Accomplishment

a) Effect of 20S oxysterol in ovo injection on developmental transcription factors at early stage of chick embryo development

Several gaps still persist in understanding of gene expression patterns of developmental markers of early embryo. We evaluated the early effect of 20(s) oxysterol (oxy) on developmental transcription factors at stage 15 chick embryo. Cobb 500 broiler breeder eggs were set in egg incubator at 99°F without rotating. After 50-55 h (stage 14-15) of incubation, window was opened in the side of the egg to expose the embryo. Eggs were exposed to one of six treatments 1) no injection no window opened, 2) no injection but window opened, 3) injection with PBS, 4) 0.08ug of oxy 5) 0.8ug of oxy 6) 1.6ug of oxy. Treatments were loaded to micro injection and 1ul of the solution was injected into vitelline vein using picospritzer III microinjection dispense system. After injection, 2 drops of pen-strep (10x) was added and the window was sealed with tape. The eggs were placed back in the incubator and 6 embryos per treatment were collected at 1h and 3h post-injection. Whole embryo was used to extract RNA, reverse transcribed to cDNA and qPCR was used to analyze expression of transcription factors at both time points. 1.6ug of oxy increased expression of Gli and Col1A2 gene at 1h post-injection. BMP2 expression was increased in embryo treated with 0.08 and 0.8ug of oxy at 3h and 1.6ug of oxy at 1h post-injection. There was no effect of oxy on ABCA1 and LBH expression at both time point of injection. Hedgehog (Hh) signaling pathway is important for development of multiple organs and bone development in embryo. BMP family is known for signaling early development in embryogenesis and bone formation. Positive feed-back loop of BMP2 plays an intermediate role in Shh-fgf4 signaling which is required for proper development of limb bud. Our finding highlights 20 (s) oxysterol could enhance the complex signaling pathway between Hh and BMP that could regulate the early patterning events in fetal skeletogenesis. Further studies needs to be conducted to understand the interaction of oxysterol with different signaling pathway of early embryo development.

b) Effects of lysophospholipid on growth performance and carcass characteristics in broilers

We evaluated the effects of supplementing different levels of lysophospholipid product (Lipidol Ultra^{TM}) to normal or reduced energy diets on growth performance and carcass characteristics in broilers. A total of 960 day old cobb 500 male birds were randomly placed to 8 treatments with 6 replicates each with 20 birds. The experiment employed a 2 × 4 factorial arrangement. Factors were energy level (normal or 100kcal/kg ME reduced) and Lipidol Ultra^{TM} level (0, 0.025%, 0.050% or 0.075%) of diets. Three diet phases were fed throughout the trial: starter (d 0-7), grower (d 8-21), and finisher (d 22-42). Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were calculated at the conclusion of each phase. At d 42, four birds per replicate were selected to measure the carcass characteristics. The birds fed with normal energy diets (NE) group had higher BW and BWG in all phases; higher FI in grower and overall trial period; and better FCR in the starter and finisher periods compared with the birds fed reduced energy diets (RE) group ($P<0.05$). Lipidol Ultra^{TM} main effects were observed with a lower FI at 0.05% dosage during the finisher period and throughout the study ($P<0.05$) and then resulted in an improved trend of cumulative FCR compared with the others ($P<0.1$). The interactions showed adding 0.075% Lipidol Ultra^{TM} to NE improved BW, BWG and FI in finisher and the overall period compared with

the one without Lipidol Ultra™ (P<0.05). In RE, improved growth performance was with 0.025% dosage (P<0.05). For the meat characteristics, NE had higher live body, hot carcass, cold carcass, major, minor, wing, and leg weight (P<0.05) but a lower minor percentage(P<0.1). The main effect of 0.075% dosage increased dressing percentage compared with the rest (P<0.05). The interactions were detected with adding 0.05% and 0.075% Lipidol Ultra™ in NE diets increased major percentage compared with the one without Lipidol Ultra™ (P<0.05). In conclusion, adding 0.025% Lipidol Ultra™ to low energy diets, and 0.075% to normal diets had a positive effect on growth performance and the latter could improve meat characteristics as well. Moreover, supplementation of 0.05% Lipidol Ultra™ would help birds balance the BWG and FI to reach a better FCR.

4. Published Papers

1. Regassa*, A., K.W. Park, and **W.K. Kim** (2016) Phenamil enhances the adipogenic differentiation of hen preadipocytes Cell Biology International 40: 1123-1128.
2. Hadley, J.A. M. Harvat-Gordon, **W.K. Kim**, C.A. Praul, D. Burns, R.M. Leach (2016) Bone sialoprotein keratan sufate proteoglycan (BSP-KSPG) and FGF-23 are important physiological components of medullary bone. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, Part A 194:1-7.
3. Norris, KM, W. Okie, **W.K. Kim**, R Adhikari*, S King, and R Pazdro. (2016) A high-fat diet differentially regulates glutathione homeostasis in the obesity-prone mouse strains DBA/2J, C57BL/6J, and AKR/J. Nutrition Research 36:1316-1324.

4. Impacts

We found that 20S oxysterol is a novel activator of Hedgehog signaling to regulate mesenchymal cell differentiation. Thus, this compound can be potentially used for intervention of obesity and osteoporosis in humans and animals. In ovo injection is an effective way to introduce bioactive molecules to embryos. Identifying time of injection, sites of injection and dosage of bioactive molecules is critical. Once identifying the best conditions, 20S oxysterol can be a highly potent molecule to induce bone and reduce fat in chicken models.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 5

The Ohio State University (Kichoon Lee)

Accomplishments:

I have been working in the area of adipocyte using cell lines, transgenic/knockout mice and food animals. My research has been focused on identifying new factors that regulate fat accretion and enhance feed efficiency that are of significant financial importance to poultry producers. However, further understanding of these genes in vivo with direct and conclusive experimental evidence requires development of transgenic poultry that express these candidate genes under the control of tissue-specific promoters. Recently, my lab identified tissue specific promoters and successfully generated, for the first time, transgenic quail expressing transgene exclusively in adipose tissue, muscle or intestine. In 2016, we also generated transgenic quail overexpressing G0S2 gene in adipose tissue and provided direct evidence that G0S2 inhibits lipolysis. Furthermore, inhibition of lipolysis in adipose tissue by G0S2 overexpression delayed egg laying, providing insight into an important mechanism of lipolysis regulation for yolk development and egg production in poultry (Int J of Mol Sci 2016).

With these accumulating resources and experiences, we continue our studies aiming to understand the role of novel genes, that we recently identified by comparative analysis of microarray, in regulating adipose or muscle accretion using transgenic bird models. In addition, the successful expression of GFP in the intestinal epithelial cells in transgenic quail under the chicken mucin 2 promoter will lead to the use of this vector system to overexpress: 1) defensin to alleviate this food safety issue by decreasing salmonella colonization, 2) bacterial-derived phytase to improve phosphorus utilization in the gut tissues, and 3) gut hormones to increase feed intake and digestion efficiency. These transgenic animals would allow producers to operate more efficiently and potentially increase production value if the GMO poultry can be approved to be included in the food chain as the GMO fish were recently approved as a source of food in Canada and US FDA. My recent publications from studies using avian transgenesis technology have been highlighted for high efficiencies and an excellent avian model for obesity research in several recent review papers.

Lastly, recent development of the clustered, regularly interspaced, short palindromic repeats (CRISPR)-Cas9 technology enables us to edit genomes in various cells and species. However, successful CRISPR/Cas9-mediated insertion/deletion (indel) mutation remains to be demonstrated in avian cells and species. We have developed a poultry-specific CRISPR/Cas9 vector to efficiently introduce targeted mutation in chromosomes of the QM7 (quail muscle clone 7) cell line. Our results showed multiple indel mutations in a certain quail locus in nearly half of the alleles being tested, suggesting the high efficiency of the system for targeted gene modification. The new CRISPR vector developed from this study has the potential application in generating knockout avian cell lines and knockout poultry.

Publications

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

1. Ahn J, Wu H, Suh Y, Carranza AC, Relling AE, Shin S, Lee SS, **Lee K**. 2017. Comparative sequence analysis and adipose-specific expression of G0S2 and ATGL in sheep. Small Ruminant Research 153 (8): 1-4.
2. Mamuad LL, Kim SH, Choi YJ, Soriano AP, Cho KK, **Lee K**, Bae GS, Lee SS. 2017. Increased propionate concentration in Lactobacillus mucosae fermented wet brewers grains and during in vitro rumen fermentation. Journal of Applied Microbiology. doi: 10.1111/jam.13475.
3. Ahn J, Park YJ, Lee TJ, Jeon Y-J, Croce CM, Suh Y, Hwang S, Kwon WS, Pang MG, Kim CH, Lee SS, **Lee K**. 2017. Comparative expression profiling of testis-enriched genes regulated during the development of spermatogonial cells. PloS One. 12(4): e0175787.
4. Ahn J, Lee J, Park JY, Oh KB, Hwang S, Lee C, **Lee K**. 2017. Targeted genome editing in a quail cell line using a customized CRISPR/Cas9 system. Poultry Science. 96 (5): 1445-1450.
5. Woodfint RM, Chen PR, Ahn J, Suh Y, Hwang S, Lee SS, **Lee K**. 2017. Identification of the MUC2 Promoter as a Strong Promoter for Intestinal Gene Expression through Generation of Transgenic Quail Expressing GFP in Gut Epithelial Cells. International Journal of Molecular Science. 18(1):E196.
6. Lee JW, Kwon DJ, Kim DW, Hwang IS, Kim DE, Kim HJ, Kim JS, **Lee K**, Im GS, Hwang S. 2017. Generation of α-1,3-Galactosyltransferase Knocked-out Transgenic Cloned Pigs with Knocked-in 5 Human Genes. Transgenic Research 26(1):153-163.
7. Biswas AA, Mamuad LL, Kim SH, Choi YJ, Avedoza CG, Bae GS, **Lee K**, Lee SS. 2016. Use of lysozyme as a feed additive on in vitro rumen fermentation and methane emission. Submitted to Asian-Australasian Journal of Animal Sciences 29(11):1601-1607.
8. Choi YM, Hwang S, **Lee K**. 2016. Comparison of muscle fiber and meat quality characteristics in different Japanese quail lines. Asian-Australasian Journal of Animal Sciences 29 (9): 1331-1337.
9. Chen PR, **Lee K**. 2016. Invited Review: Inhibitors of myostatin as methods of enhancing muscle growth and development. Journal of Animal Science. 94(8):3125-3134.
10. Shin S, Ahn J, Suh Y, Moeller SJ, Hwang S, **Lee K**. 2016. Isolation and in vitro validation of cardiac muscle-specific promoters in pigs. Cellular and Molecular Biology. 62: 123.
11. Song KH, Kwak CH, Jin UH, Ha SH, Park JY, Abekura F, Chang YC, Cho SH, **Lee K**, Chung TW, Ha KT, Lee YC, Kim CH. 2016. Housekeeping promoter 5'pcmah-2 of pig CMP-N-acetylneuraminic acid hydroxylase gene for NeuGc expression. Glycoconjugate Journal. 3:779-788.
12. Choi YM, Chen PR, Shin S, Zhang J, Hwang S, **Lee K**. 2016. Mild heat stress enhances differentiation and proliferation of Japanese quail myoblasts with slow muscle fiber characteristics. Poultry Science. 95 (8):1912-1917.
13. Ha SW, Lee JM, Kwon KM, Kwak CH, Abekura F, Park JY, Cho SH, **Lee K**, Chang YC, Lee YC, Choi HJ, Chung TW, Ha KT, Kim CH. 2016. Exogenous and endogeneous disialosyl ganglioside GD1b induces apoptosis of MCF-7 human breast cancer cells. International Journal of Molecular Science. 17(5):E652.
14. Chen PR, Shin S, Choi YM, Kim E, Han JY, **Lee K**. 2016. Overexpression of G0/G1 Switch Gene 2 in Adipose Tissue of Transgenic Quail Inhibits Lipolysis Associated with Egg Laying. International Journal of Molecular Science. 17(3):E384.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 6

University of Arkansas for Medical Sciences (Brian Piccolo)

1. **Impact Nugget.**

The Physioanalytics Lab at UAMS is focused on integrating multiple omics based technologies to understand and uncover novel physiology in various systems. Much of the focus has been centered on utilizing metabolomics to identify amino acid and other metabolic patterns in several rodent models of energy utilization. Increasingly, the gut microbiome is being assessed to uncover relationships among gut bacteria and circulating or tissue metabolism.

2. **New Facilities and Equipment.**

NONE.

3. **Unique Project Related Findings.**

Skeletal muscle amino acid concentrations are decreased after exercise in skeletal muscle specific transgenic mice.

Liver amino acids concentrations are decreased and correlated to dietary resistant starch intake in mice fed a high fat diet.

Branched chain amino acids are significantly increased during the progression of diabetes in the UC Davis Type-2-Diabetes Mellitus Rat Model in contrast to significant decreases of virtually all other amino acids and amino acid derivatives.

4**. Accomplishment Summaries.**

Novel findings include evidence that UCP3 may influence skeletal muscle tissue amino acid metabolism under conditions of exercise, the gut microbiome may influence hepatic amino acid metabolism, and that branched chain amino acids appear to be a consequence of diabetes development rather than causative.

5. Impact Statements.

Most of the studies from this lab have been descriptive, but setting the stage for more targeted and nuanced approaches to investigate observations from these studies. Furthermore, we've been developing HTML based bioinformatic tools to assist and increase the efficiency of our data analysis pipeline.

6. Published Written Works.

Books

None

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Book Chapters

None

Refereed Journal Articles

1. Aguer CA, P**iccolo BD,** Fiehn O, Adams SH, Harper ME, "A novel amino acid and metabolomics signature in mice overexpressing muscle uncoupling protein 3," *FASEB J*, 2017 Feb;31(2):814-827. doi: 10.1096/fj.201600914R. Epub 2016 Nov 10.
2. Kieffer DA, **Piccolo BD**, Marco ML, Kim EB, Goodson ML, Keenan MJ, Dunn TN, Kudsen, KEB, Martin RM, Adams SH, "Mice fed a high-fat diet supplemented with resistant starch display marked shifts in liver metabolome concurrent with altered gut bacteria," *J Nutr*, 2016 Dec; 146(12):2476-2490. Epub 2016 Nov 2 pii: jn238931. doi: 10.3945/jn.116.238931. Epub 2016 Nov 2.
3. Kieffer DA, **Piccolo BD**, Marco ML, Kim EB, Goodson ML, Keenan MJ, Dunn TN, Kudsen, KEB, Adams SH, Martin RM, "Obese mice a diet supplemented with enzyme-treated wheat bran display marked shifts in the liver metabolome concurrent with altered gut bacteria," *J Nutr*, 2016 Dec; 146(12):2445-2460. doi: 19:10.3945/jn.116.238923. Epub 2016 Oct 19
4. **Piccolo BD,** Graham JL, Stanhope KL, Fiehn O, Havel PJ, Adams SH, "Plasma amino acid and metabolite signature tracking diabetes progression in the UCDT2D Rat model of type 2 diabetes," *Am J Physiol Endocrinol Metab,* 2016 Jun 1;310(11):E958-69. doi:10.1152/ajpendo.00052.2016. Epub 2016 Apr 19.

Symposium Proceedings

None

Poster Presentations

1. Bhattacharyya S, **Piccolo B**, Mercer K, Chintapalli, Shanker K, Yeruva L, "Integrative analysis of microbiome and metabolome of the porcine gut reveals diet-associated interrelationship," Showcase of Medical Discoveries: Biomedical Informatics Research: Little Rock, Sept. 2016

Popular Articles

None

Other Creative Works

None

Scientific and Outreach Oral Presentations

1. **Piccolo B**, Bowlin A, Saraf M, Mercer K, Bhattacharya S, Chintapalli S, Adams S, Shankar K, Badger T, Yerva L, "Early Diet has Differential Effects on the Small Intestine Microbiome by Region in Neonatal Piglets," Experimental Biology, Chicago, IL, April 2017.
2. **Piccolo B**, Graham J, Wankhade U, Nookaew I, Shankar S, Havel P, Adams S, "Host Diabetes Status is the Major Regulator of Gut Microbiome in the UCD-T2DM Rat," Experimental Biology, Chicago, IL, April 2017.

3. Reubel M, **Piccolo B,** Moutos D, Shankar K, Andres A, "Untargeted Metabolomics Reveal Disparate Metabolite Profile in Follicular Fluid between Obese and Normal Weight Women," Experimental Biology, Chicago, IL, April 2017.
4. Mercer K, Saraf M, **Piccolo B**, Sharma N, Yeruva L, "Effects of early cholesterol intake on cholesterol 7 alpha hydroxylase (Cyp7a1) expression in piglets receiving sow's breast milk or infant formula until weaning. Keystone Symposia – Bile Acid Receptors as Signal Integrators in Liver and Metabolism (C1), Monterey, CA, March 2017.
5. Bhattacharyya S, Sharma N, Pack L, **Piccolo B**, Yeruva L, Mercer KE, "Changes in liver metabolomics profile in piglets fed with formula milk versus breast milk," Colloquium on Emerging Metabolomics, Las Vegas, NV, July 2016.

7. Fund leveraging.

None.

8. Other relevant accomplishments and activities.
None.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 7

University of Tennesse (Brynn Voy)

1. Accomplishments

Objective 1: Share and critique new techniques, experimental designs, and in progress-recent data. The Voy lab at the University of Tennessee continues to focus on the impact of diet on adipose deposition in juveniles, using broiler chicks as a model organism. In the past year we shifted focus to the maternal diet, to investigate developmental programming of adiposity and its potential role in childhood obesity. We completed a study in which hens were fed diets containing fish oil or corn oil, and the impact on adipose development in chicks was evaluated. The manuscript describing this study is under revision for Journal of Nutrition, and the results were presented at EB and Poultry Science in 2016. We also began a collaborative effort to investigate the role of adipose fatty acid mobilization in adapting to lactation stress in beef cattle.

Objective 2: Increasingly implement newly emerging high through-put omics based technologies and experimental methodologies. We continue to work with the Campagna lab at UTK to develop and implement methods for metabolomic profiling of adipose tissue. In 2016 we began to develop a method to measure acyl-amino acids, which have been implicated in metabolic regulation. Preliminary data indicate that fasting progressively increases the abundance of these modified amino acids in adipose tissue and serum. Follow-on experiments are underway to further characterize these metabolites in broiler chicks. We also used LC-MS/MS-based proteomics in 2016 to identify proteins in chick adipose tissue that were altered by maternal consumption of fish oil. These results are included in the manuscript below that is under revision for Journal of Nutrition.

2. Publications.

1. Torchon E, Ray R, Hulver MW, McMillan RP, Voy BH. Fasting rapidly increases fatty acid oxidation in white adipose tissue of young broiler chickens. Adipocyte, 6(1):33-39 [epub ahead of print], 2016.

2. Howard, SJ. Effects of dietary fatty acids on adipose development in broiler chicks. Master's Thesis, University of Tennessee, 2016. http://trace.tennessee.edu/utk_gradthes/4290

3. Impacts.

Our finding that maternal consumption of fish oil reduces adiposity in offspring highlights a new avenue and set of pathways through which adipose tissue deposition can potentially be controlled through the maternal diet.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Report 8
Purdue University (Kola Ajuwon)

1. **Impact Nugget.**

Purdue University determined the impact of acute water and feed stress in newly weaned piglets.

Purdue University determined the impact of garlic compounds in the remediation of stress and inflammation in pigs.

2. **New Facilities and Equipment.**

NONE.

3. **Unique Project Related Findings.**

Purdue University determined the effect of dietary supplementation of exogenous multi-enzyme mixture containing carbohydrases and phytase on growth performance, energy and nutrient digestibility, and selected mucosal gene expression.

Purdue University determined effect of threonine deficiency on intestinal integrity and immune response to feed withdrawal combined with coccidial vaccine challenge in broiler chicks.

Purdue University determined impact of gestational calorie intake in sows regulates early postnatal adipose tissue development in the offspring

4**. Accomplishment Summaries.**

The major findings this year relate to discoveries on the impact of garlic compounds in lessening stress and inflammatory markers in pigs. In addition, we determined the impact of threonine on intestinal integrity in birds challenged with coccidia. We also determined the molecular basis of maternal calorie over consumption during pregnancy on offspring performance and tissue characteristics.

5. Impact Statements.

The findings show that maternal calorie over consumption program the offspring for possible obesity development later in life. Adequate threonine supplementation is necessary for optimal performance of birds during disease challenge.

6. Published Written Works.

Books

None

Book Chapters

None

Refereed Journal Articles

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

1. Horn, N.L., F. Ruch, C.R. Little, G. Miller, K.M. Ajuwon and O. Adeola. 2016. Impact of acute feed and water deprivation at weaning and subsequent heat stress on growth performance and ileal morphology in nursery pigs. *J. Anim. Sci*. 94: supplement 3: 289-293.doi:10.2527/jas.2015-9849.
2. Horn, N.L., F. Ruch, C.R. Little, G. Miller, K.M. Ajuwon and O. Adeola. 2016. Expression of cytokine and tight junction genes and ileal mucosal morphology in nursery pigs in response to garlic diallyl disulfide and diallyl trisulfide compounds. *J. Anim. Sci*. 94: supplement 3: 40-44. doi:10.2527/jas.2015-9718.
3. Horn, N.L., F. Ruch, C.R. Little, G. Miller, K.M Ajuwon and O. Adeola. 2016. Determination of the adequate dose of garlic diallyl disulfide and diallyl trisulfide for effecting changes in growth performance, total-tract nutrient and energy digestibility, ileal characteristics, and serum immune parameters in broiler chickens. *Poult. Sci. 95:2360-5. doi: 10.3382/ps/pew126.*
4. Xue, P., K.M. Ajuwon and O. Adeola. 2016. Phosphorus and nitrogen utilization responses of broiler chickens to dietary crude protein and phosphorus levels. *Poult Sci. 95:2615-2623.*
5. Cowieson, A.J., H. Lu, K. M. Ajuwon, I. Knap and O. Adeola. 2016. Interactive effects of dietary protein source and exogenous protease on growth performance, immune competence and jejunal health of broiler chickens. *Anim. Prod. Sci*. http://dx.doi.org/10.1071/AN15523.
6. Ajuwon, K. M., E.J. Arentson-Lantz and S. S. Donkin. 2016. Excessive gestational calorie intake in sows regulates early postnatal adipose tissue development in the offspring. *BMC Nutrition. 2:29*.
7. Lu. H., A. Preynat, V. Legrand-Defretin, P. A. Geraert, O. Adeola and K.M. Ajuwon. 2016. Effects of dietary supplementation of exogenous multi-enzyme mixture containing carbohydrases and phytase on growth performance, energy and nutrient digestibility, and selected mucosal gene expression in the small intestine of weanling pigs fed nutrient deficient diets. *Can. J. Anim. Sci*. 96: 243-251, 10.1139/cjas-2015-0078.
8. Zhang, Q., X. Chen, S.D. Eicher, K.M. Ajuwon, and T.J. Applegate. 2016. Effect of threonine deficiency on intestinal integrity and immune response to feed withdrawal combined with coccidial vaccine challenge in broiler chicks. *Br. J. Nutr.* 116:2030-2043. doi: 10.1017/S0007114516003238.
9. Adeola, O., Xue, P., A. J. Cowieson and K. M. Ajuwon. 2016. Basal endogenous losses of amino acids in protein nutrition research for swine and poultry. *Anim. Feed. Sci. Tech.* 221:274–283 *http://dx.doi.org/10.1016/j.anifeedsci.2016.06.004*
10. Huang. C.W., Y. J. Chen, J. T. Yang, C.Y. Chen, K.M. Ajuwon, S.E. Chen, N.W. Su, Y.S. Chen, H.J. Mersmann, and S.T. Ding. 2017. Docosahexaenoic acid increases accumulation of adipocyte triacylglycerol through up-regulation of lipogenic gene expression in pigs. *Lipids Health Dis.* 16(1):33. doi: 10.1186/s12944-017-0428-3.
11. Lu, H., H. Yan, M. G. Ward, T. Stewart, O. Adeola, and K. M. Ajuwon. 2017. Effect on Rendement Napole (RN) genotype on metabolic markers in Ossabaw pigs fed different levels of fat. *J. Anim. Physiol. Anim. Nutr*. *(Berl). In Press*

Symposium Proceedings

Fleisher, D.H., H. Baruh and K.C. Ting. 2001. Model-based predictive control for biomass production in advanced life support. Proceedings of the 2nd IFAC-CIGR Workshop on Intelligent Control for Agricultural Applications, Bali, Indonesia. August 22-24. pp. 198-203.

Station Reports
NCCC210 Mtg., Chicago 2017
Brian Piccolo (Chair), Woo Kyun Kim (Co-Chair)

Poster Presentations

1. *Ajuwon, K.M., and H. Qu. 2016.* Increased intracellular calcium is a mediator of heat stress induced adipocyte hypertrophy. Presented at the 2016 Experimental Biology meeting. *FASEB J*. 30:915.27.
2. Qu, H. and K.M. Ajuwon. 2016. Heat stress induces distinct lipidomic profile in differentiating porcine adipocytes. Presented at ASAS 2016 meeting. *J. Anim. Sci.* 94, E-Suppl. 5.
3. Ajuwon, K. M and V. V. Almeida. 2016. Fiber type consumed in early life affects future obesity susceptibility and metabolic health in the Ossabaw pig model of diet-induced obesity. Presented at The Power of Programming 2016. International Conference on Developmental Origins of Adiposity and Long-Term Health, Munich, Germany, October 13 – 15, 2016.

Popular Articles

None

Other Creative Works

None

Scientific and Outreach Oral Presentations

1. Lu. H., H. Yan, H. M. Masey O'Neill, C. L. Bradley, M.R, Bedford, P. Wilcock, C. H. Nakatsu, O. Adeola and K. M. Ajuwon. 2016. Effect of timing of post-weaning supplementation of xylanase on growth performance, nutrient digestibility and fecal microbial composition in weanling pigs. Presented at ASAS 2016 meeting. *J. Anim. Sci.* 94, E-Suppl. 5.
2. Lu. H., H. Yan, H. M. Masey O'Neill, C. L. Bradley, M.R, Bedford, P. Wilcock, C. H. Nakatsu, O. Adeola and K. M. Ajuwon. 2016. Effect of xylanase and live yeast supplementation on growth performance and gut microflora diversity of growing pigs. Presented at ASAS 2016 meeting. *J. Anim. Sci.* 94, E-Suppl. 5.
3. Yan. H and K.M. Ajuwon. 2016. Butyrate increases tight junction protein expression and enhances tight junction integrity in porcine IPEC-J2 cells stimulated with LPS. Presented at ASAS 2016 meeting. *J. Anim. Sci.* 94, E-Suppl. 5.
4. Almeida, V. V. and K.M. Ajuwon. 2016. Effect of prior fiber consumption on diet-induced obesity susceptibility and metabolic health indicators in Ossabaw pigs. Presented at ASAS 2016 meeting. *J. Anim. Sci.* 94, E-Suppl. 5.
5. Beeson, L.A., C. L. Walk, P. Hastie, M. R. Bedford, O. Adeola, K.M. Ajuwon and O. A. Olukosi. 2016. Manipulation of dietary phytic acid, myo-inositol and exogenous phytase levels influenced the blood insulin to glucose ratio but not their overall concentration. Presented at *2016 P*SA Annual Meeting, July 11–14, New Orleans, Louisiana*. Poult. Sci.* 95(E-Suppl. 1).
6. Zhang, Q., S. D. Eicher, K.M. Ajuwon and T. J. Applegate. 2016. Effect of threonine deficiency on intestinal integrity and immune response to coccidiosis in broiler chicks. Presented at *2016* PSA Annual Meeting, July 11–14, New Orleans, Louisiana*. Poult. Sci.* 95(E-Suppl. 1).
7. Ajuwon, K.M. 2016. Zinc, Impact on Gut Immunity and the Microbiome-Implication for Growth and Nutrient Utilization Efficiency in Animals. Presented at *The 4th* International Forum on Micronutrient and *Feed* Safety conference. September 22-24, 2016, Changsha, China.

8. Lu, H., N. L. Horn, O. Adeola, and K.M. Ajuwon. 2017. Understanding Gut Development in the Pig and Implication for Health and Nutrient Utilization. Proceedings. Presented at the Manitoba Swine Seminar, Feb 1-2, 2017. Vol. 31:153-167.

7. Fund leveraging.

None.

9. Other relevant accomplishments and activities.
None.