Station Reports
NCCC210 Mtg., San Diego 2017

General Highlights

NCCC210 Meeting – April 20, 2018

- Opened meeting at 9:30
- Attendees: Woo Kyun Kim, Werner Bergen, Sean Adams, Theo van Kempen, Kichoon Lee, Stone Ding, Jim Kinder, Brynn Voy and one student, Eric Testroet, Kim Barnes
- Discussion about meeting location – suggestion to send email to all members to get feelings on EB without ASN before making decision. Comments about the lack of students at this year's meeting. Some discussion about Nutrition-Physiology group providing the basic science nutrition, which may pull more nutrition back to EB even without ASN. Similar situation when ASBMB pulled out of EB many years ago. ASN currently has a 3-year commitment to be outside of EB. Proposed conference call after ASN meeting to reassess the better location mid-late June (Jim will be in Australia). Possible to allow people to "attend" electronically using Skype or Zoom technologies.
- Renewal – need to emphasize the NCCC committee goals of sharing information. Emphasize the NCCC purpose – then expand on how we do that. New objectives – like to see some variation from one proposal to the next. Request to renew due Sept 15, objectives by Oct 15, Appendix E by Nov 15, full proposal by Dec 1.
- Everyone needs to send Woo their individual station reports so he can submit the final report within 60 days.
- Co-chair for 2019/chair 2020 will be Eric Testroet.
- There is currently no NIFA rep listed – Jim said to email Chris to ask about this, also to ask about access to submit the renewal.
- Presentations from the Tennessee (Voy), Nutreco (van Kempen), Ohio (Lee), National Taiwan University (Ding), Georgia (Kim), Arkansas (Adams), Washington (Testroet), and Alabama (Bergen) stations.

1. Auburn University
2. Werner G. Bergen and Terry D. Brandebourg
3. Accomplishment

The effect of a compensatory growth strategy and feeding omega 3 fatty acids on porcine carcass composition at harvest.

We continue to assess differential expression (DE) of lipogenic and lipolytic/ oxidative genes in adipose (s.c.) tissue and skeletal (loin) muscles of finishing Yorkshire pigs. Two production trials were conducted. In the first trial the complete finisher period was divided into an initial dietary N restriction (to invoke compensatory gain) coupled with poultry fat (2%) and flaxseed oil supplementation (3% flaxseed oil on DM basis) followed by a final finisher phase at100 % nitrogen requirements and continued poultry fat and flaxseed oil supplementation. All pigs were harvested at the end of the final finisher phase and adipose tissues and loin muscle tissues samples were obtained. In a second production trial, pigs were finished in a two phase trial, first at 17.5 % followed by 16 % dietary protein respectively. In a factorial design, poultry fat was added to the phase 1 and 2 diets at 0, 2, 4, 6 % and vitamin E at 11 and 220 IU/kg. Finally all but the control diet in trial two were supplemented with 1% flaxseed oil. All pigs were harvested at the end of the final finisher phase and adipose tissues and loin muscle tissues samples were obtained. The swine production trials were conducted in cooperation with our swine nutrition group led by Dr. L.I. Chiba.

The hypothesis for these 2 production trials was that a short term restriction of lysine and/or higher protein levels in early finisher diets may somehow enhance intramuscular fat formation while feeding fat and an omega-3 fatty acid source would lower depot adipose tissue lipogenesis. Vitamin E was supplemented to combat potential linolenic acid oxidation.

The flaxseed is a rich 18:3 omega 3 source. In rodents, supplementation of omega three PUFA lowers de novo fatty acid synthesis in the liver. Since in pigs there is no de novo fatty acid synthesis in the liver but in adipose tissues, we were interested whether the omega 3 fatty acid supplementation would affect lipogenic and fatty oxidative gene DE from the non-omega 3 supplemented pigs. Finally swine carcass 18:3 omega 3 fatty acid content in adipose and muscle tissues was assessed using chloroform: methanol extraction of tissues followed by gas chromatography of long chained fatty acids.

The dietary treatments had no effect on intramuscular fat in finished pigs in both production trial.

Differential Gene Expression was conducted for fatty acid synthase (FASN), glycerol phosphate dehydrogenase (GPDH), stearyol CoA desaturase (SCD), PAAR-gamma 2, sterol response element binding protein-1c (SREBP-1c), PPAR- alpha, leptin and

adiponectin. Dietary supplementations of poultry fat, flaxseed and/or Vitamin E had no effect on expression of these lipid metabolism genes in either of the two production trials. Flaxseed supplementation increased muscle 18:3 omega 3 content (P<.05).
Impacts: Supplementations of omega 3 PUFA (mostly linolenic acid) and extra fat to swine diets did not result in changes in marbling and expression of enzymes and regulatory trans factors involved in lipid synthesis. Feeding of 1 % flaxseed did however significantly increase the linoleic acid contend of muscle foods without effecting organoleptic properties of cuts of meat. The potential of pork products from pigs on flaxseed supplemented diets as a satisfactory source of omega 3 PUFA in the human diet has not been evaluated.
Papers published:
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 198:182-190.

Bergen WG.2017. Peptide-Based Regulation of Metabolism as Related to Obesity. Endocrinology. 2017 Oct 1; 158(10):3081-3082. doi: 10.1210/en.2017-00696.

Hausman GJ, **Bergen WG**, Etherton TD, Smith SB. 2018. The history of adipocyte and adipose tissue research in meat animals. J Anim Sci. 96:473-486 . doi: 10.1093/jas/skx050.

The Brandebourg Laboratory:

Two overriding issues define our research program: 1) the general failure of meat animals to reach their potential for optimal growth and carcass merit, and 2) the emergence of obesity as a global health epidemic. As such, our work focuses upon the regulation of fat cell differentiation, molecular drivers of feed efficiency and the establishment of a swine model for human metabolic disease. A common thread is the impact that adipose tissue biology has on these issues.
The Mangalica pig was imported to Auburn University for use as a novel model to study the underlying mechanisms linking obesity and diabetes and to better understand adipose tissue development, the regulation of feed intake, and to leverage as novel swine model of extreme marbling. We have established that Mangalica pigs display an extreme, early onset, morbidly obese phenotype that is driven by a voluntary energy intake that exceeds their metabolic needs by roughly 2 to 3-fold. Furthermore, when becoming obese, similarly to humans, these animals, 1) develop chronic inflammation based upon expression of tissue markers, circulating cytokine levels, and responses to endotoxin challenges, 2) display an obesogenic, inflammatory microbiota, and 3)

exhibit metabolic dysregulation. Therefore, this breed displays great potential to serve as a relevant animal model of obesity-induced metabolic syndrome and as a unique model to better understand the regulation of satiety, adipose tissue development and energy balance in food animals.

Creation of transmitochondrial Mangalica and Yorkshire experimental animals

We are currently extending this model by pursuing a mitochondrial-nuclear DNA exchange strategy whereby mitochondria (and thus the inherent mtDNA) will be switched between embryos of lean, efficient and obese, inefficient pigs (Yorkshires and Mangalicas, respectively) in order to investigate the role of mitochondrial-nuclear crosstalk in the downstream inflammatory and metabolic dysregulation associated with obesity. The mtDNA exhibits significant geographic variation in different populations of vertebrates, represented as mtDNA "haplogroups". Studies show that mitochondrial DNA directly influences metabolism and susceptibility to cardiovascular disease in rodents and that mitochondrial DNA exchange confers protection against cardiovascular disease in obese mice with mitochondria transferred from genetically lean counterparts. Specifically, our collaborators at the University of Alabama at Birmingham have directly linked mtDNA haplogroup with susceptibility to develop heart failure using Mitochondrial Nuclear eXchange (MNX) mice. However, there are no studies that directly test the hypothesis that mtDNA background holds significant influence over adiposity or obesity-induced inflammation and metabolic disease. Importantly, we have recently characterized polymorphisms between the mtDNA of Mangalicas and Yorkshire pigs suggesting that differences in mitochondrial function could exist between these breeds.

We hypothesize that mtDNA from lean pigs could protect obese pigs from obesity-associated metabolic complications. If correct, our resulting novel, translational model would have the potential to greatly impact obesity research by revealing new genetic mechanisms which give rise to obesity and metabolic disease. Importantly, these pigs could also serve as a useful model to study the role of the mitochondria on productive performance, body composition, and meat quality. Thus, we are currently extending the MNX technique from the mouse to the pig by validating collection and culture conditions and *in vitro* fertilization protocols to produce viable porcine trans-mitochondrial embryos for implantation into surrogate dams.

Characterization of mitochondrial variation between oocytes harvested from lean and obese pigs.

Our overall aim is to examine the role of mitochondrial in obesity-induced metabolic disease and in production efficiency/meat quality in food animals. Thus, we've conducted a pilot study to first verify that differences in oocyte mitochondria exist between lean and obese pigs. This was accomplished by characterizing the number and distribution of mitochondria and quantifying lipid content in porcine oocytes harvested

from post-pubertal lean Yorkshire and obese Mangalitsa females. To this end, oocytes were aspirated from the ovaries of cycling, unsynchronized pigs. Oocytes were stained with brilliant cresyl blue (BCB) to predict developmental competence. BCB+ staining indicates higher developmental competence. BCB+ oocytes were then stained with MitoView Green fluorescent stain to visualize mitochondrial number and distribution. Finally, BCB+ oocytes were denuded and stained with lipophilic Nile Red to determine lipid content. Upon staining, 77% of Yorkshire oocytes and 88% Mangalitsa oocytes were BCB+. Furthermore, BCB+ Mangalitsa oocytes had higher corrected total cell fluorescence and, thus, higher numbers of mitochondria than BCB+ Yorkshire oocytes or either group of BCB- oocytes ($P < 0.05$). Mangalitsa oocytes had higher contents of both polar ($P < 0.05$) and neutral lipids ($P < 0.05$) compared with Yorkshire oocytes (and a higher ratio of polar to neutral lipids within individual oocytes). These results suggest that differences do exist between the mitochondria of Yorkshire and Mangalitsa pigs, consistent with our hypothesis that disparities in mitochondria account in part for their divergent body composition. These data support further pursuit of mitochondrial nuclear exchange between Mangalitsa and Yorkshire pigs to discover whether manipulation of the mitochondrial genome can uncouple obesity from metabolic disease.

Impacts: If successful this work will lead to a novel swine model to study the role of the mitochondria in productive efficiency and meat quality that could also serve as a translational model for human obesity and metabolic disease.

Papers published:

Hunter-Beasley, E. N., C. R. Kerth, C. L. Bratcher, L. K. Anderson, **T. D. Brandebourg**, and C. W. Rowe. 2018. Ractopamine Hydrochloride Did Not Impact Carcass Traits, Muscle Fiber Types, or Sensory Traits of Long-Fed Yearling Heifers. Meat and Muscle Biology 2:83-91.

Meloche, K.J., Dozier, W.A., **Brandebourg, T.D**. and J.D. Starkey. 2018. Skeletal muscle growth characteristics and myogenic stem cell activity in broiler chickens affected by wooden breast. *Accepted* Poultry Science (doi pending)

Patents and Inventions:

Harrison Moss and **T.D. Brandebourg**. 2017. U.S. Patent Application 483.1-100 “Animal Health Monitoring Device and System”.

1. Washington Station Report
2. Min Du
3. Accomplishments

During the last year, we are studying the impacts of vitamin A administration to neonatal calves on the intramuscular adipose tissue and growth performance of resulting steers. In addition, we are exploring underlying mechanistic changes, for which an in vitro experimental model is needed. Therefore, we established a 3-dimensional spheroid culture system for studying adipose tissue development of beef cattle. Briefly, stromal vascular cells derived from bovine intramuscular fat were isolated and stored in liquid nitrogen before culturing. Cells were cultured in hanging drops for 3 days to allow for the formation of spherical structures. The spheroids were then transferred to cell culture plates with endothelial basal medium-2 (EBM-2) for 3 days and in DMEM supplemented with a standard adipogenic cocktail for 3 additional days, which were then allowed to fully differentiate for 3 days in DMEM supplemented with insulin. Compared with conventional 2-dimensional culture, cells in a 3-dimensional spheroid culture system had higher adipogenic gene expression and consequently contained more adipocytes with larger lipid droplets. In addition, endothelial induction of spheroids prior to adipogenic differentiation is essential for efficient induction of adipogenesis of bovine stromal vascular cells, mimicking in vivo adipose development. In summary, the newly developed 3-dimensional spheroid culture method is an efficient way to induce adipogenic differentiation and study adipose development of cells derived from ruminant animals, which also can be used for studying the role of angiogenesis in adipose development.

Journal Articles:

Zhang, S., Y. Zhang, X. Zhou, X. Fu, J. Michal, G. Ji, M. Du, and Z. Jiang. (2018). Alternative polyadenylation drives genome-to-phenome information detours in the AMPKα1 and AMPKα2 knockout mice. Scientific Report, Accepted.

Li, T., J. Gao, M. Du, J. Song, and X. Mao. (2018). Milk fat globule membrane attenuates high-fat diet-induced obesity by inhibiting adipogenesis and increasing uncoupling protein 1 expression in white adipose tissue of mice, Nutrients, In press.

Zou, T., B. Wang, Q. Yang, J. M. de Avila, M. J. Zhu, J. You, D. Chen, and M. Du. (2018). Raspberry promotes brown and beige adipocyte development in mice fed high-fat diet through activation of AMP-activated protein kinase (AMPK) a1. Journal of Nutritional Biochemistry, In press.

Gao, J., J. Song, M. Du, and X. Mao. (2018). Bovine a-lactalbumin hydrolysates (a-LAH) ameliorate adipose insulin resistance and inflammation in high-fat-diet-fed C57BL/6J mice. Nutrients, In press.

Ma, Y.N., B. Wang, Z.X. Wang, N. A. Gomez, M. J. Zhu, and M. Du. (2018). Three dimensional spheroid culture of adipose stromal vascular cells for studying adipogenesis in beef cattle. Animal, In press.

Sun, X., X. Fu, M. Du, and M. J. Zhu. (2018). Ex vivo gut culture for tracing gut epithelial development. Open Biology, 8: 170256.

Sun, X., M. Du, D. A. Navarre and M. J. Zhu. (2018). Purple potato extract promotes intestinal epithelial differentiation and barrier function by activating AMP-activated protein kinase. Molecular Nutrition and Food Research, In press.

Maricelli, J. A., Y. M. Bishaw, B. Wang, M. Du, and B. D. Rodgers. (2018). Systemic SMAD7 gene therapy increases striated muscle mass and enhances exercise capacity in a dose-dependent manner. Human Gene Therapy, In press.

Bibi, S., M. Du, and M. J. Zhu. (2018). Dietary red raspberries reduces colorectal inflammation and carcinogenic risk in DSS-induced colitis in mice. Journal of Nutrition, In press.

Zhu, M. J., Kang, Y., Y. Xue, X. Liang, M. P. Gonzalez Carcia, D. Rodgers, D. K. Kagel, and M. Du. (2018). Red raspberries suppress NLRP3 inflammasome and attenuate metabolic abnormalities in diet-induced obese mice. Journal of Nutritional Biochemistry, 53:96-103.

Bibi, S., Y. Kang, M. Du, and M. J. Zhu. (2018). Dietary red raspberries attenuate dextran sulfate sodium-induced acute colitis. Journal of Nutritional Biochemistry, 51:40-46.

Xing, T., Y. Kang, X. Xu, B. Wang, M. Du, and M. J. Zhu. (2018). Raspberry supplementation improves insulin signaling and promotes brown-like adipocyte development in white adipose tissue of obese mice. Molecular Nutrition and Food Research, 2018, 62:1701035.

Son, J. S., S. A. Chae, E. D. Testroet, M. Du, and H. Jun. (2018). Exercise-induced myokines: a brief review of controversial issues of this decade. Expert Review of Endocrinology & Metabolism, 13: 51-58.

Chen, Y., Y. Liu, M. Du, W. Zhang, L. Xu, X. Gao, L. Zhang, H. Gao, L. Xu, J. Li, M. Zhao. (2017). Constructing a comprehensive gene co-expression based interactome in Bos Taurus. PeerJ, 5: e4107.

Wang, B., X. Fu, X. Liang, J. M. Deavila, Z. Wang, L. Zhao, Q. Tian, J. Zhao, N. A. Gomez, S. C. Trombetta, M. J. Zhu, and M. Du. (2017). Retinoic acid induces white adipose tissue browning by increasing adipose vascularity and inducing beige adipogenesis of PDGFRa+ adipose progenitors. Cell Discovery, 3:17036.

Fu, X., Q. Yang, B. Wang, J. Zhao, M. Zhu, S. M. Parish, and M. Du. (2017). Reduced satellite cell density and myogenesis in Wagyu compared to Angus cattle as a possible explanation of its high marbling. Animal, 9: 1-8.

Gao, P. F., X. H. Guo, M. Du, G. Q. Cao, Q. C. Yang, Z. D. Pu, Z. Wang, Q. Zhang, M. Li, Y. S. Jin, X. J. Wang, H. Liu, and B. G. Li. (2017). LncRNAs profiling of skeletal muscles in Large White pigs and Mashen pigs during development. Journal of Animal Science, 95: 4239-4250.

Li, T., X. Cheng, M. Du, B. Chen, and X. Y. Mao. (2017). Upregulation of heme oxygenase-1 mediates the anti-inflammatory activity of casein glycomacropeptide (GMP) hydrolysates in LPS-stimulated macrophages. Food & Function, 8: 2475-2484.

Wang, B., Z. Wang, J. M. de Avila, M. J. Zhu, F. Zhang, N. A. Gomez, L. Zhao, Q. Tian, J. Zhao, J. Maricelli, H. Zhang, B. D. Rodgers, and M. Du. (2017). Moderate alcohol intake induces thermogenic brown/beige adipocyte formation via elevating retinoic acid signaling. FASEB Journal, 31: 4612-4622.

Wang, B., X. Fu, M.J. Zhu, and M. Du. (2017). Retinoic acid inhibits white adipogenesis by disrupting GADD45A mediated Zfp423 DNA demethylation. Journal of Molecular Cell Biology, 9: 338-349.

Zhao, J., Y. Jin, M. Du, W. Liu, Y. Ren, C. Zhang, J. Zhang. (2017). The effect of dietary grape pomace supplementation on epididymal sperm quality and testicular antioxidant ability in ram lambs. Theriogenology, 97: 50-56.

Xue, Y., M. Du, H. Sheng, C. J. Hovde, and M. J. Zhu. (2017). Escherichia coli O157:H7 suppress host autophagy and promote bacterial adhesion via Tir-mediated and cAMP-independent activation of protein kinase A. Cell Death and Discovery, 3, 17055.

Zhao, J. X., R. Su, W. Liu, Y. Ren, C. Zhang, M. Du, and J. Zhang. (2017). Effect of dietary Tartary buckwheat extract supplementation on growth performance, meat quality and antioxidant activity in ewe lambs. Meat Science, 134: 79-85.

Zhao, J., Q. Yang, L. Zhang, X. Liang, X. Sun, B. Wang, Y. Chen, M. J. Zhu, and M. Du. (2017). AMPKa1 deficiency suppresses brown adipogenesis in favor of fibrogenesis during brown adipose tissue development. Biochemical and Biophysical Research Communications, 491: 508-514.

Du, M., S.P. Ford, and M. J. Zhu. (2017). Optimizing livestock production efficiency through maternal nutritional management and fetal developmental programming. Animal Frontiers, 7: 5-11.

Sun, X. X. Fu, Q. Y. Yang, M. Du, and M. J. Zhu. (2017). AMPK regulate intestinal differentiation via histone modification of CDX2. Cell Death and Differentiation, 24: 819-831.

Xue Y., M. Du, and M. J. Zhu. (2017). Quercetin suppresses NLRP3 inflammasome activation in epithelial cells triggered by Escherichia coli O157:H7. Free Radical Biology & Medicine, 108: 760-769.

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) a1 in mice fed high-fat diet. Molecular Nutrition and Food Research, 61: 1600746.

Song, J.J., Q. Wang, M. Du, T. G. Li, B. Chen, X. Y. Mao. (2017). Casein glycomacropeptide-derived peptide IPPKKNQDKTE ameliorate high glucose-induced insulin resistance in HepG2 cells via activation of AMPK signaling. Molecular Nutrition and Food Research, 61: 1600301.

Yang, G., S. Bibi, M. Du, T. Suzuki, and M. J. Zhu. (2017). Regulation of the intestinal tight junction by natural polyphenols: a mechanistic perspective. Critical Reviews in Food Science and Nutrition, 595: 1547-1562.

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, 18:288-299.

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, 7:43716.

Griner, J. D., C. J. Rogers, M. J. Zhu, and M. Du. (2017). Lysyl oxidase propeptide promotes adipogenesis through inhibition of FGF-2 signaling. Adipocyte, 6: 12-19.

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.

Li, T., B. Chen, M. Du, J. Song, X. Cheng, X. Wang, and X. Mao. (2017). Casein glycomacropeptide hydrolysates exert cytoprotective effect against cellular stress by upregulating HO-1 expression in HepG2 cells. Nutrients, 9: E31.

Xue, Y., S. Zhang, M. Du, and M. J. Zhu. (2017). Dandelion extract suppresses reactive oxidative species and inflammasome in intestinal epithelial cells. Journal of Functional Food, 29: 10-18.

Station Reports
NCCC210 Mtg., San Diego 2017

1. National Taiwan University

2. Shih-Torng Ding

3. Accomplishments

Our lab has focused on establishing animal models for human disease such as fatty liver, liver fibrosis, obesity and diabetes. We used adult laying hens to identify biomarkers for NAFLD and indicated that AACS, DPP4, GLUL, and GST could be considered to be potential diagnostic indicators for NAFLD in the future. This research has been awarded with **Milton Sundae Award** by American Nutrition Science Association. We also successfully established a NASH pig model, and our findings suggested an association of NASH with ER stress and autophagy. On the nutritional effect on adipocytes, we found that the effect of DHA to promote adipogenesis to trap TAG in adipocytes and also increase expression of genes involved in adipocyte fatty acid oxidation. Our results suggest a direct effect of DHA on adipocyte metabolism, resulting in TAG turnover and fatty acid dissipation to facilitate plasma lipid uptake from the circulation. Our efforts on regulation of SOAT1 gene from avian species has great results that show glucose, glucagon, and IBMX can regulate the expression of this gene, suggesting that possible regulation mechanisms can be utilized to modify the development of avian embryos.

1. Tsai, M.T., Y.J. Chen, C.Y. Chen, M.S. Tsai, C.L. Han, Y.J. Chen, H.J. Mersmann and **S.T. Ding (corresponding)**. 2017. Identification of potential plasma biomarkers for non-alcoholic fatty liver disease by integrating transcriptomics and proteomics in laying hens. J. Nutr. 147:293-303. doi: 10.3945/jn.116.240358. (SCI, NUTRITION & DIETETICS, 16/81, IF:4.145)

2. Tsai, M.T., N. Ohbashyashi, K. Iwasaki, N. Ohkohchi, **S.T. Ding, Y. Kanaho, and Y. Funakoshi**. 2017. Regulation of HGF-induced hepatocyte proliferation by the small GTPase Arf6 through the PIP_2-producing enzyme PIP5K1A. *Scientific Reports* **7**, Article number: 9438. doi:10.1038/s41598-017-09633-z.

3. Wu, C.Y.; H.Y. Liu, C.W. Huang, S.Y. Yeh, N.C. Cheng, S.T. Ding **(co-corresponding)**, and H.Y. Chen. 2017. Synergistically controlled stemness and multilineage differentiation capacity of stem cells on multifunctional biointerfaces. Adv. Materials Inter. 4: e1700243. (Materials Science, Multidisciplinary, 47/275, IF=4.279).

4. Liu, H.Y., C.C. Chen, Y.Y. Lin, Y.J. Chen, B.H. Liu, S.C. Wong, C.Y. Wu, Y.T. Chang, H.Y. E. Chou and **S.T. Ding (corresponding).** 2017. Chitosan-assisted differentiation of porcine adipose tissue-derived stem cells into glucose-responsive insulin-secreting clusters. PLOS ONE. 2017. 12(3):e0172922. (SCI, MULTIDISCIPLINARY SCIENCES Top 20, 15/64, IF:3.394)

5. 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 (corresponding)**. 2017. Docosahexaenoic acid increases accumulation of adipocyte triacylglycerol through up-regulation of lipogenic gene expression in pigs. Lipids Helth. Dis. **DOI:** 10.1186/s12944-017-0428-3. (SCI, Nutrition and Dietetics, 52/81, IF=2.073)

6. Chang, Y.M., **S.T. Ding**, E.C. Lin, L.A. Wang, and Y.W. Lu. 2017. A microfluidic chip for rapid single nucleotide polymorphism (SNP) genotyping using primer extension on microbeads. Sens. Actuator B-Chem. 246: 215-224. (SCI, INSTRUMENTS & INSTRUMENTATION Top 5%, 2/58, IF:5.401)

7. Liu, F.W. S.T. Ding E.-C. Lin, Y.W. Lu, and J.S.R. Jang. 2017. Automated melting curve analysis in droplet microfluidics for single nucleotide polymorphisms (SNP) genotyping. RSC Advances 7:4646-4655. (SCI, Chemistry, Multidisplinary, 59/166, IF:3.108)
8. Lin, H.J., Y.S. Chen, Y.J. Chen, Y.Y. Lin, H.J. Mersmann, and **S.T. Ding (corresponding)**. 2017. Modulation of fatty acid oxidation and glucose uptake by oxytocin in adipocytes. J. Biomed. Sci. Eng. 10:37-50. https://doi.org/10.4236/jbise.2017.102005.
9. Peng, Y.J. T.L. Shen, Y.S. Chen, H. J. Mersmann, B.H. Liu, and S.T. Ding. 2018. Adiponectin and adiponectin receptor 1 overexpression enhance inflammatory bowel disease. J. Biomedical Science. **25**:24. https://doi.org/10.1186/s12929-018-0419-3
10. Li, H.J., H.J. Lin, S.T. Ding, and C.Y. Chen. 2018. he role of pericardial adipose tissue in the heart of obese minipigs. European Journal of Clinical Investigation. In Press.

1. Ohio State University

2. Kee C. Lee

3. Accomplishments

Development of genome-editing technology in avian by our unique CRISPR-Cas9. 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 the development of poultry with gene knockout. 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 quail. Our results showed multiple indel mutations in a specific target gene in 16 out of 28 allele in vitro (57%) and 5 out of 11 quail lines in vivo (45%), suggesting the high efficiency of our system for targeted gene modification. The new CRISPR vector developed from my laboratory will greatly impact the area of adipocyte biology, since we are targeting new adipose-specific genes that were recently discovered in my laboratory to understand their functions in vivo. We believe our studies will contribute to advance poultry science and benefit industry, expecting more discoveries, research grants, and commercialization in the near future.

Paper published.

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 153:1-4.
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. 123:29-40.

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.

1. West Virginia University

2. Kimberly Barnes

3. Accomplishment

The focus of our laboratory has been on dietary supplementation of various specific fatty acids, conjugated linoleic acid (CLA) and omega-3, on lipid metabolism and adipose tissue biology. We have established that supplementation of CLA to mice that have been fed coconut oil will result in a greater reduction in body fat and increased lipolysis, as compared to mice supplemented CLA after being fed soy oil. This is accompanied by a reduction in adipocyte-specific gene expression and alterations in fatty acid metabolites. We have reported increased degree of saturation of fatty acids and in ketone body concentrations. We also have data on metabolite profiles in the liver of mice fed the different diets, with greater differences between mice fed soy oil vs coconut oil than mice fed CLA or not. We are currently summarizing data generated by several students over a number of years on this project into a manuscript, which is being prepared by Master's student Megan Nugent. Our other focus has been on omega-3 fatty acid sources. We have demonstrated that mice have greater absorbance of dietary DHA from fish oil compared to algae oil. This appears to be related to the greater percent of DHA in fish oil being localized at the sn-2 position on the dietary TAG than in algae oil, where the DHA is more evenly distributed between esterification positions. This results in less DHA (and total omega-3) incorporation into tissues. Fish oil is also more effective at reducing serum lipids in mice fed a standard chow diet than algae oil, even when tissue levels of DHA were similar. This project is also undergoing additional statistical analyses and summary into manuscript form. Due to Dr. Barnes's increased administrative role no new data has been generated in the past year.

1. Purdue University

2. Kolapo Ajuwon

3. Accomplishment

Our focus in 2017 continued to be understanding the mechanism involved in the regulation of adipose and intestinal tissue development and function. In addition, we continue to conduct research in the area of effects of fiber and fiber products on metabolism and function of intestinal tissue. A major finding in 2017 was the role of butyrate in the regulation of epithelial cell function. Using the IPEC-J2 porcine intestinal cell model, our results showed that butyrate enhanced epithelial cell growth and proliferation, and expression of tight junction proteins, via upregulation of AKT signaling pathway. Another important finding was through a collaborative work with Shingo Kajimura, where we showed existence of UCP1 independent thermogenesis in adipocytes through enhanced calcium signaling. Additionally, using pigs fitted with jugular catheters, we demonstrated the time-course of release of inositol into the blood of pigs given phytase and inositol. Given the importance of inositol and phosphorus in metabolism, this work provides fundamental information on time-dependent release of inositol and other metabolites into the blood stream of phytase-fed pigs. In other projects, we described the impact of the RN genotype on metabolic response of Ossabaw pigs fed diets with different levels of fat.

Publications:

1. 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.
2. 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).* 2018 102(1):e132-e138. doi: 10.1111/jpn.12720.
3. Yan, H., and K. M. Ajuwon. 2017. Butyrate enhances intestinal barrier function in IPEC-J2 cells through a selective upregulation of tight junction proteins via the Akt signaling pathway. PLoS One. 2017 12(6):e0179586. doi: 10.1371/journal.pone.0179586.
4. Zhang, Q., X. Chen, S.D. Eicher, K.M. Ajuwon, and T. J. Applegate. 2017. The effect of threonine on secretory immune system using a chicken intestinal *ex vivo* model with lipopolysaccharide challenge. *Poul. Sci.* 96:3043-3051. doi: 10.3382/ps/pex111.
5. Zhang, Q., S.D. Eicher, K.M. Ajuwon, and T. J. Applegate. 2017. Development of a chicken ileal explant culture model for measurement of gut inflammation induced by lipopolysaccharide. *Poul. Sci.* 96:3096-3103. doi: 10.3382/ps/pex160.
6. Ikeda, K., Q. Kang, J.P. Camporez, H. Maki, M. Homma, K. Shinoda, T. Yoneshiro, S. Altshuler-Keylin, P. Maretich, K. M. Ajuwon, B. M. Spiegelman, T. Soga, and S. Kajimura. 2017. UCP1-independent thermogenesis and glucose metabolism in beige fat through calcium cycling. *Nat. Med.* 23:1454-1465. doi: 10.1038/nm.4429.
7. Cowieson, A. J., F.F. Roos, J.P. Ruckebusch, J.W. Wilson, P. Guggenbuhl, H. Lu, K.M. Ajuwon, and O. Adeola. 2017. Time-series responses of swine plasma metabolites to ingestion of diets containing myo-inositol or phytase. *Br. J. Nutr*. 118:897-905. doi: 10.1017/S0007114517003026.
8. Horn. N., G. Miller, K.M. Ajuwon, and O. Adeola. 2017. Ability of garlic-derived diallyl disulfide and diallyl trisulfide supplemented by oral gavage to mitigate effects of an acute postweaning feed and water deprivation event in nursery pigs*. J. Anim. Sci.* 95:3579-3590. doi: 10.2527/jas.2017.1545.

9. Horn, N., G. Miller, K.M. Ajuwon, and O. Adeola. 2017. Garlic diallyl disulfide and diallyl trisulfide mitigates effects of pro-oxidant induced cellular stress and has immune modulatory function in LPS-stimulated porcine epithelial cells. *J. Anim. Sci*. 95:4045-4051. doi: 10.2527/jas2017.1546.
10. Ma, D., Y.H.B. Kim, B. Cooper, J.H. Oh, H. Chun, J.H. Choe, J.P. Schoonmaker, K. Ajuwon, and B. Min. 2017. Metabolomics profiling to determine the effect of postmortem aging on color and lipid oxidative stabilities of different bovine muscles. *J. Agric. Food. Chem.* 65:6708-6716. doi: 10.1021/acs.jafc.7b02175.

1. University of Tennessee

2. Brynn H. Voy

3. Accomplishment

Objective 1: Share and critique new techniques, experimental designs, and in progress---recent data. In 2017 my lab shared new data about an ongoing project that addresses developmental programming of adipose biology through maternal dietary fat during the annual project meeting, which was held in Chicago. My graduate student, Ronique Beckford, gave the presentation and benefitted from the group's usual lively discussion and insightful questions.
Objective 2: increasingly implement newly emerging high through---put omics based technologies and experimental methodologies. In 2017, we used RNAseq to characterize the adipose transcriptomes of chicks hatched from hens that were fed diets supplemented with corn oil or fish oil. These data provided the basis for a chapter in Dr. Beckford's dissertation and for a manuscript that is in its final stages of review. We also continued to use LC---MS---based metabolomics to comprehensively profile phospholipids, acylcarnitines and small molecule metabolites in tissue extracts, including adipose tissue.
Objective 3: Publish peer---reviewed scientific reviews. We submitted a review article that is pending publication in Current Metabolomics, in which we describe the status of using transcriptomics and metabolomics for studies of poultry.

1. Publications.
1. Beckford RC, Howard SJ, Das S, Farmer AT, Campagna SR, Yu J, Hettich RL, Wilson JL, Voy BH. Maternal consumption of fish oil programs reduced adiposity in broiler chicks. Sci Rep. 7(1):13129, 2017.

2. Clemmons, B.A., Mihelic, R.I., Beckford, R.C., Powers, J.B., Melchior, E.A., McFarlane, Z.D., Cope, E.R., Embree, M.M., Mulliniks, R.T., Campagna S.R., Voy, B.H., and Myer, P.R. Serum Metabolites Associated with Feed Efficiency in Black Angus Steers. Metabolomics. 13 (12), 147, 2017.

3. Torchon, Emmanuelle, R. C. Beckford, S. Das, and B. H. Voy. Enriching the starter diet in fish oil reduces adipocyte size in broiler chicks. Current Developments in Nutrition, 1 (11), 2017.

4. Beckford, R, E. Tague, S. Campagna, and B. H. Voy. Transcriptomic and Metabolomic Profiling of Chicken Adipose Tissue: Dual Purpose Benefit for Human Obesity and Poultry Production, Current Metabolomics, in press.

5. 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, 2017.

2. Impacts.

As in past years, the project meeting in 2017 provided ample opportunity for our lab to exchange and discuss data with other group members. The RNAseq data that we generated under this project in 2017 have spawned new hypotheses about lipid mediators and their roles in adipocyte development. These data are being used as the basis for grant proposals that will be submitted in 2018.

1. University of Georgia

2. Woo Kyun Kim

3. Accomplishments

a) The effects of different fatty acids on the expression of adipogenic transcripts and adipogenic differentiation in chicken preadipocytes

This study was conducted to examine effects of different fatty acids (FAs) with/without chicken serum (CS) on the expression of adipogenic transcripts and adipogenesis in hen preadipocytes. In experiment 1, preadipocytes were grown in DMEM containing 10% FBS (Control) and treated with 300 μM oleic acid (OA)+FBS, linoleic acid (LA)+FBS, palmitic acid (PA)+FBS, or stearic acid (SA)+FBS for 48hr. In experiment 2, cells were grown in 1X DMEM containing 5% CS and treated with 300 μM OA (CS+OA), PA (CS+PA), SA (CS+SA) or 200 μM LA (CS+LA) for 48hr. Adipogenesis was determined using Oil Red O staining. The proportion of OA, PA, or SA was increased ($P<0.05$) in preadipocytes grown in either FBS or CS and treated with OA, PA or SA. Except FBS+SA, adipogenesis was induced in FBS+OA, FBS+LA, FBS+PA, CS+OA, CS+LA, CS+PA, CS+SA compared to FBS. Compared to FBS cells, the expression of FABP4 mRNA increased ($P<0.05$) in FBS+OA, FBS+LA, or FBS+PA, whereas that of C/EBPα, C/EBPβ, and ATGL increased ($P <0.05$) in FBS+OA or FBS+LA cells only. Expression of FABP4 and C/EBPβ mRNA was higher ($P<0.05$) in CS, CS+OA, CS+LA, CS+PA, or CS+SA compared with the control (FBS) cells, whereas the expression of ATGL and C/EBPα was higher ($P<0.05$) in CS, CS+OA, or CS+LA compared with FBS cells. In conclusion, these results showed that FAs have different potentials to induce key adipogenic transcripts and adipocyte formation, LA is the most potent among the tested FAS, and these adipogenic potentials can be improved in the presence of CS.

b) Potent anti-adipogenic effect of green tea extracts in chicken. This study was conducted to examine the effect of green tea on body, adipose tissue, and liver weights and adipose tissue weight to body weight ratio and the adipogenic differentiation and expression of adipogenic transcripts in chicken (*Gallus gallus*) preadipocytes. In experiment one, chicks were weighed and randomly assigned to a control diet (CTRL) and CTRL + 1% (w/w) green tea powder for 38 days. In experiment 2, preadipocytes were isolated from 20 wk old

chicken and treated with an adipogenic cocktail (DMIOA) containing 500 nM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 20 µg/mL insulin, and 300 µM OA (DMIOA), DMIOA+30 µg of extract B, E, H, Mc, T, and W, respectively, for 48 h. Data were analysed using the GLM procedure of the Statistics Analysis System (SAS) Institute version 9.4, and differences were considered significant at $P < 0.05$. Gene expression was measured using real-time PCR. Green tea supplementation reduced ($P < 0.05$) total body, liver and abdominal fat weight and the expression of key adipogenic transcripts, such as FABP4, C/EBPβ, and C/EBPα and adipogenic differentiation of chicken preadipocytes. These results demonstrate that supplementation of green tea could be an effective strategy in the control of obesity in chickens.

4. Published Papers

1. Wang, G., **W.K. Kim**, M.A. Cline, and E.R. Gilbert (2017). Factors affecting adipose tissue development in chickens: A Review. Poultry Science (In print).
2. Regassa*, A., M. Suh, J. Datar, C. Chen*, and **W. K. Kim** (2017) Fatty acids have different adipogenic differentiation potentials in stromal vascular cells isolated from abdominal fat in laying hens. Lipids 52:513-522.
3. Regassa*, A., D. Lee, S Choi, C Song, and **W. K. Kim** (2017) Potent anti-adipogenic effect of green tea extracts in chicken. Journal of Diabetes and Obesity 4:1-6.
4. Regassa*, A., E. Kiarie, J.S. Sands, M.C. Walsh, **W.K. Kim**, and C.M. Nyachoti (2017) Nutritional and metabolic implications of replacing corn starch with D-xylose in broiler chickens fed corn and soy bean meal based diet. Poultry Science 96:388-396.
5. 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.
6. Regassa*, A., K.W. Park, and **W.K. Kim** (2016) Phenamil enhances the adipogenic differentiation of hen preadipocytes Cell Biology International 40: 1123-1128.

5. Impact

Avian can be a good model for lipid metabolism and adipogenesis research. Through this model, it has been found that various fatty acids have different adipogenic potential to regulate lipid accumulation and fat cell formation. Green tea containing anti-adipogenic compounds showed body weight control in fast growing chicken model, suggesting the extracts from green tea can be weight control substances for human as well.