ANNUAL PROGRESS REPORT
NATIONAL RESEARCH SUPPORT PROJECT – NRSP008
Year Ending 2004
Preliminary Information-Not for Publication

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I. PROJECT TITLE:

NRSP008 : National Animal Genome Research Program

II. COOPERATING AGENCIES AND PRINCIPAL LEADERS:

A. Agencies and Departments Cooperating: North Carolina Research and Experiment Station and Animal Science Department, North Carolina State University

B. Leaders of the Project: Joe Cassady (leader) and Melissa Ashwell

III. NATURE OF WORK AND PRINCIPAL RESULTS OF YEAR:

Objective 1: *Enhance and integrate genetic and physical maps of agriculturally important animals for cross species comparisons and sequence annotation.*

Nature of work: Characterize novel single nucleotide polymorphisms (SNP) in commercial lines of pigs.
Principal Results: Tissue samples have been collected from 96 sires (24 from each breed) representing American Duroc, Yorksire, Hampshire, and Landrace breeds. Extensive pedigree searches were conducted to identify sires, within breeds, which were as unrelated as possible. Extraction of DNA is complete and preliminary DNA sequencing to identify SNPs has been done.

Objective 2: *Facilitate integration of genomic, transcriptional, proteomic and metabolomic approaches toward better understanding of biological mechanisms underlying economically important traits.*

Nature of work: The objective of this study was to characterize changes in allelic frequencies for two RFLPs associated with the *follistatin* gene in a line of pigs selected for increased litter size (LS).

Principal Results:

The LS line was selected for increased number of fully formed pigs, and litters were standardized at birth so replacement gilts were reared in litters of ten or fewer pigs. A contemporary control line (C) was maintained. In generation nine, estimated mean breeding values for litter size differed between lines by 0.63 pigs ($P < 0.01$). *Follistatin*, a cysteine-rich, glycoprotein encoded by a single gene, was investigated. Based on expression patterns and implications from studies involving *follistatin* function, it can be concluded that *follistatin* may play an important role in determining litter size. Intronic regions were amplified using PCR, and two RFLPs, characterized by *Msp*I (*FS1*), and *Fnu*4HI (*FS2*), were identified. Frequencies of the *B* allele of *FS1* increased in LS, and allele frequencies differed between LS and C by 0.25 and 0.18 in generations 10 and 11, respectively. Changes in allele frequency for *FS1* differed from 0 in generations 10 ($P < 0.01$) and 11 ($P < 0.057$). Standard errors were adjusted to determine if random drift could be excluded as the cause of changes in allele frequency, and differences were retested ($P < 0.29$) and ($P < 0.31$), respectively. Results for *FS2* were similar to those of *FS1*. Additive effect of the *B* allele of *FS1* on estimated breeding value for pigs born live in LS ($n = 207$) was +0.09. Marker-assisted selection has potential to be highly advantageous in selection for lowly heritable and sex-limited traits, such as litter size. Changes in allele frequency to the exclusion of random drift were not detected; however, sufficient evidence exists to support further investigation of *follistatin* as a candidate gene for litter size in pigs.

Nature of work: The objective of this project identify genes associated with adipose metabolism.

Principal Results:

We analyzed gene expression during t10c12-CLA-induced body fat reduction in a polygenic obese line of mice. Adult mice (N=185) were allotted to a 2 x 2 factorial experiment consisting of a non-obese (ICR-control) and an obese (M16-selected) line of mice fed a 7% fat, purified diet containing either 1% linoleic acid (LA) or 1% t10c12-CLA. Body weight (BW) gain by day 14 was 12% lower in CLA compared to LA fed mice ($P < 0.0001$). By day 14, t10c12-CLA reduced weights of epididymal, mesenteric and brown adipose tissues, as a percentage of BW, in both lines by 30, 27 and 58%, respectively, and increased liver weight/BW by 34% ($P < 0.0001$). Total RNA was isolated and pooled (4 per tissue•day) from epididymal adipose (day 5 & 14) of the obese mice to analyze gene expression profiles using Agilent mouse oligo microarray slides representing >20,000 genes. Numbers of genes differentially expressed by ≥ 2 fold in epididymal adipose (day 5 & 14) were 29 and 125, respectively. Of particular interest in adipose, CLA putatively increased expression of uncoupling proteins (1 and 2), carnitine palmitoyltransferase system, tumor necrosis factor-α ($P < 0.05$) and caspase-3, but decreased expression of the peroxisome proliferators activated receptor-γ, glucose transporter-4, perilipin, caveolin-1, adiponectin, resistin and bcl-2 ($P < 0.01$). In conclusion, this experiment has revealed candidate genes that will be useful in elucidating mechanisms of adipose metabolism.

Objective 3: *Facilitate and implement bioinformatic tools to extract, analyze, store and disseminate information.*

None

IV. APPLICATION OF FINDINGS:

None of these experiments have been completed at this time. Therefore any discussion regarding application of findings would be premature.

V. WORK PLANNED FOR NEXT YEAR:

Nature of work: Determination of the role of imprinted genes in porcine embryo survival. Funding has been obtained to conduct research to identify imprinted genes in the pig associated with embryo survival. This project in a collaboration among North Carolina State University College of Veterinary Medicine, College of Agriculture and Life Sciences, and USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska.

Nature of work: Identification of SNPs segregating within purebred commercial lines of pigs will continue pending the availability of funding.

VI. PUBLICATIONS:

Holl, J. W., J. P. Cassady, D. Pomp, R. K. Johnson. A genome scan for quantitative trait loci and imprinted regions affecting reproduction in pigs. Journal of Animal Science. 82(12): 3421-9, 01 Dec 2004

Blowe, C. D., E. J. Eisen, O. W. Robison, and J. P. Cassady. 2004. Characterization of a line of pigs selected for increased litter size for two RFLPs identified in *follistatin.* Abstract. J. Anim. Sci Supplement 1.

Walker, S. E., O.W. Robison, C.S. Whisnant, and J.P. Cassady. Effect of divergent selection for testosterone production on testicular morphology and daily sperm production in boars. Journal of Animal Science. 82(8):2259-63, 01 Aug 2004