Retrospective Review
National Program 101
Food Animal Production

Vision: Furnish the scientific community and the food animal industries with scientific information, biotechnologies, and best management practices that ensure consumers an abundant supply of competitively priced animal products which enhance human health, ensure domestic food security and enhance the efficiency, competitiveness and economic and environmental sustainability, of the food animal industries.

Agenda

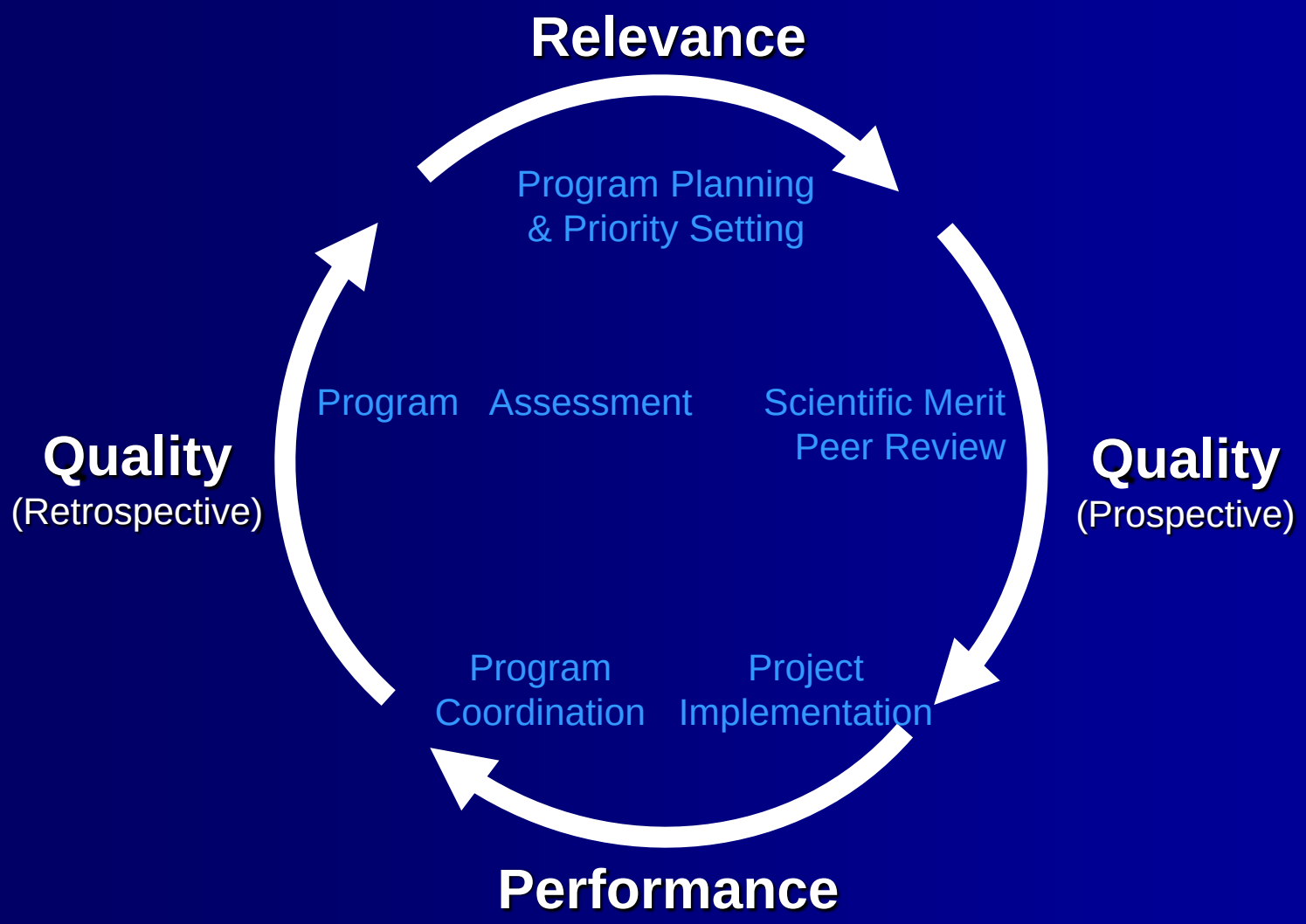
- Welcome and Charge
  - Steve Kappes, Deputy Administrator, Animal Production and Protection
- Introductions
  - Caird Rexroad, Acting National Program Leader, Food Animal Production
  - Deb Hamernik, University of Nebraska, Panel Chair
- Program Information
  - Caird Rexroad
- Accomplishment Stories: Component 1
  - ARS Scientists
- Break (15 minutes)

Agenda *continued*

- Program Summary Information
  - Caird Rexroad
- Accomplishment Stories: Components 2 and 3
  - ARS Scientists
- Panel Discussion (Panel Only)
- Panel Report and Debrief (Panel, ONP)

ARS National Program Cycle

OMB Research & Development Investment Criteria



Retrospective Analysis

- Focus on 5 years preceding completion of 3rd year cycle;
  - 2010 – 2014
  - Final 2 years of previous cycle, first 3 years of current cycle
- Conducting an in-house program assessment and documenting research accomplishments and/or progress on the National Program Action Plan for presentation to external reviewers;
- Conducting an external review of accomplishments and/or progress, based on the preceding documentation, focused on the research's relevance, quality, and impact;
- Recording the results of the review; and
- Informing ARS leadership of evaluation results.

Interactive Approach

- A PowerPoint document is prepared for presentation to the Evaluation Panel
  - Summary Information
  - Program Linkages
  - Accomplishment Examples
- Permits direct interactions between panel members and ARS Scientists, National Program Leaders, providing an opportunity for improved communication
- The presentation is supplemented by other documentation provided prior to the Panel Meeting
  - Online > Documents > Presentation
- Effectively Reduce reviewer time in reviewing and travel

Program Linkages

USDA Strategic Goal 1 Area: Nutrition, Food Safety and Quality

Goal 1.3: Enhance the economic viability and competitiveness of U.S. agriculture by maintaining the quality of harvested agricultural commodities or otherwise enhancing their marketability, meeting consumer needs, developing environmentally friendly and efficient processing concepts, and expanding domestic and global market opportunities through the development of value-added food and nonfood technologies and products, except energy and fuels

USDA Strategic Goal Area 2: Natural Resources and Sustainable Agricultural Systems

Goal 2.4 – effectively and safely manage and use manure and other agricultural and industrial byproducts in ways that maximize their potential benefits while protecting the environment and human and animal health.

Goal 2.5 - Develop and transfer economically viable and environmentally sustainable production and conservation practices, technologies, plant materials and integrated management strategies, based on fundamental knowledge of ecological processes, that conserve and enhance the Nation's diverse natural resources found on its range, pasture, hay and turf lands; and

Goal 2.6 – Develop integrated solutions to solve challenges related to agricultural system productivity, profitability, energy efficiency, and natural resource stewardship.

2012 – 2017 USDA ARS Strategic Plan Area 4

- **Goal 4.1:** Provide scientific information and biotechnologies to enhance management practices that will ensure an abundant supply of competitively priced animal and aquaculture products.

- **Performance Measure 4.1.2:** Develop new technologies and tools contributing to improved precision animal production systems to meet current and future food animal production needs of diversified consumers, while ensuring economic and environmental sustainability and animal well-being.

National Program Action Plan Research Components

- Component 1: Improving Production and Production Efficiencies and Enhancing Animal Well-Being and Adaptation in Diverse Food Animal Production Systems

- Component 2: Genetic Improvement - Understanding, Improving, and Effectively Using Animal Genetic and Genomic Resources

- Component 3: Measuring and Enhancing Product Quality and Enhancing the Healthfulness of Meat Animal Products

Component 1: Improving Production and Production Efficiencies and Enhancing Animal Well-Being and Adaptation in Diverse Food Animal Production Systems

- Problem Statement 1A: Improving the Efficiency of Growth and Nutrient Utilization
- Problem Statement 1B: Reducing Reproductive Losses
- Problem Statement 1C: Enhancing Animal Well-Being and Reducing Stress

Improved nutrient efficiency of beef cattle and swine

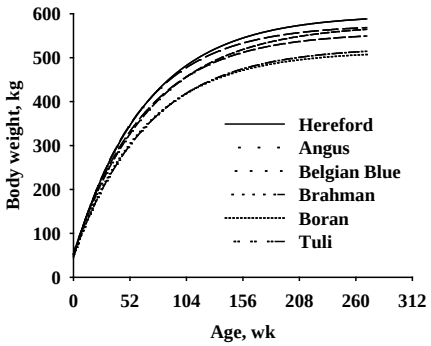
- Feed costs are the single largest input in both beef and swine production
- Less than 20% of the feed energy is converted to edible product

<u>Hypotheses</u>

- There is genetic variation in the efficiency that feed is converted to product
- Management strategies can be used to improve nutrient utilization

There is genetic variation in the efficiency that feed is converted to product

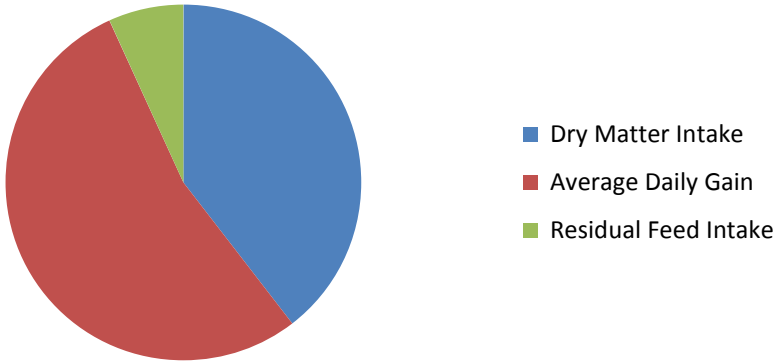
- Determined feed efficiency and its component traits in cattle are heritable using quantitative genetics



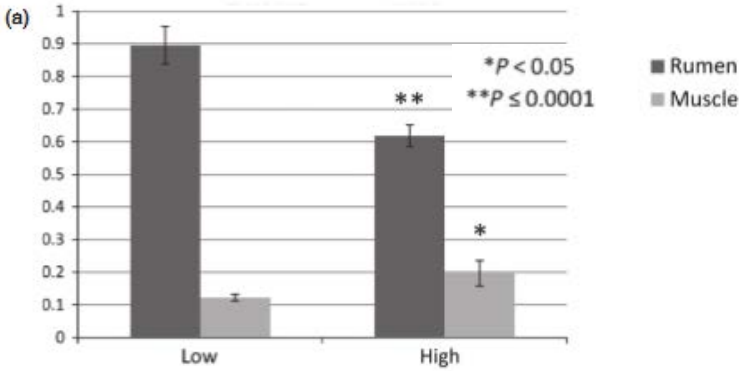
- Described genetic differences in growth patterns of beef cattle

- Identified genes and genetic markers associated with feed efficiency in beef cattle

Proportion of SNP associated with feed efficiency traits (N=220)



AMPK phosphorylated protein to total AMPK protein levels in steer rumen and muscle tissues (Western blot).



Higher levels of active AMPK in rumen of LOW DMI animals

Management strategies can be used to improve nutrient utilization

- Determined that feed coproducts (distillers grains and glycerol) can be substituted for more expensive feed ingredients. Titration studies were used to define optimum feeding levels.

- Determined there is an alternative antimicrobial to antibiotics that:
  - improves growth and feed efficiency in swine (lysozyme)
  - Improves GI tract health
  - Reduces pathogen shedding

RANGE SHEEP PRODUCTION EFFICIENCY

Problem

- Neonatal diarrhea reduces growth rates of lambs through weaning
- Pathogenic bacteria from the lambing environment is the primary cause
- Treatments are costly and some may result in antibiotic resistance

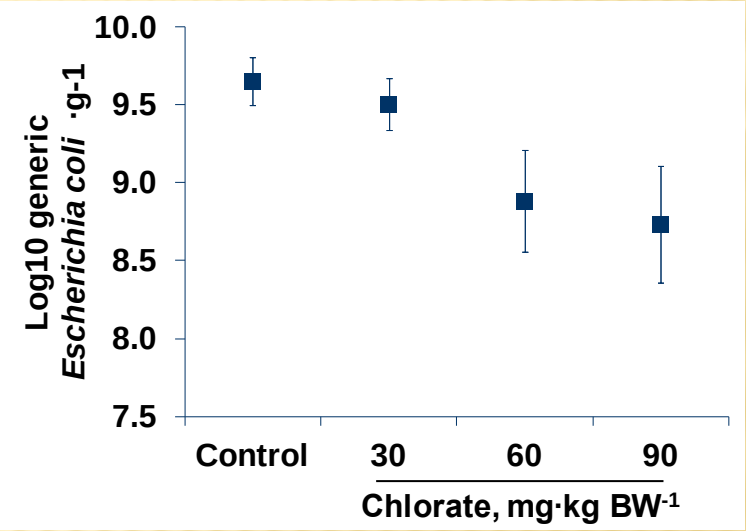
Solution

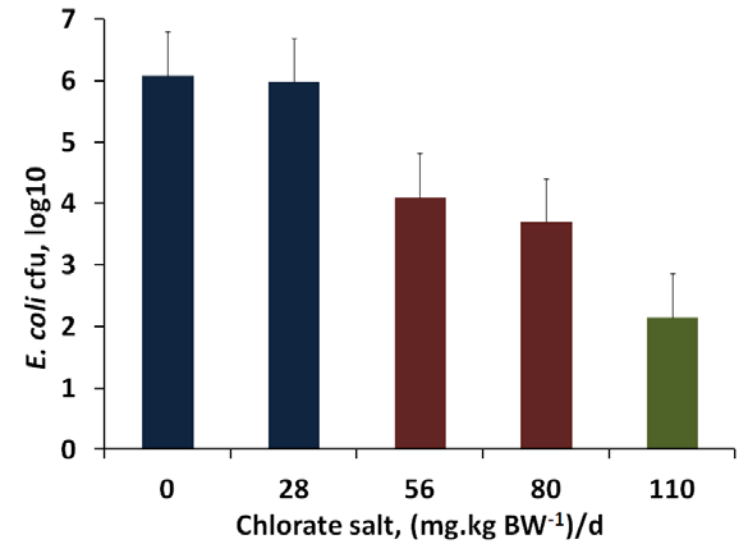
- Affordable and safe prophylactic approaches that do not cause antibiotic resistance

RANGE SHEEP PRODUCTION EFFICIENCY

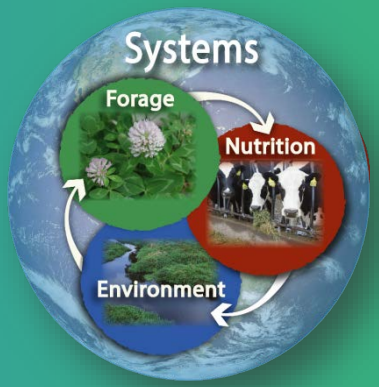
Accomplishment

- Lambs fed chlorate salts at birth had a 90% reduction in intestinal bacteria known to cause neonatal diarrhea
- Chlorate salts added to the drinking water of ewes at lambing resulted in a 99% reduction in fecal *E. coli* that was shed in the lambing environment
- Depending on the production system, elimination of neonatal diarrhea in lambs could result in a 1 to 5% increase in total lamb-crop weight at weaning

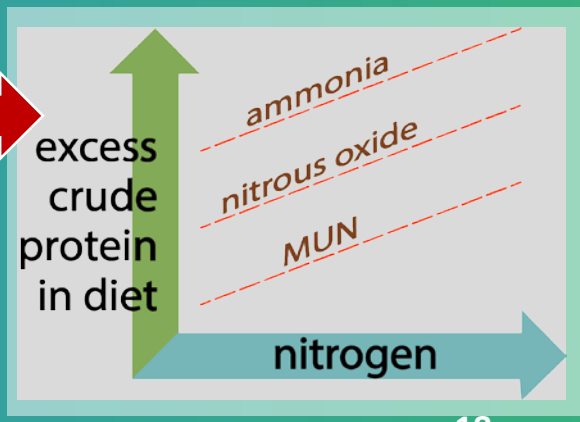




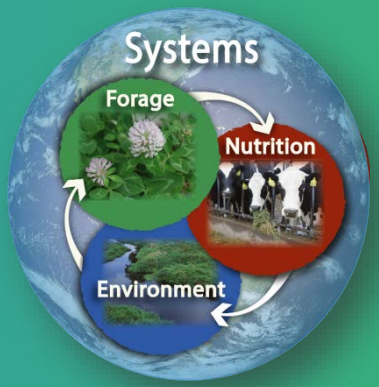
One Key to Sustainable Milk Production:



Put more protein (nitrogen) & energy in milk, not manure, practices that are both environmentally and economically sustainable



Determined Benchmarks for Nitrogen Use Efficiency

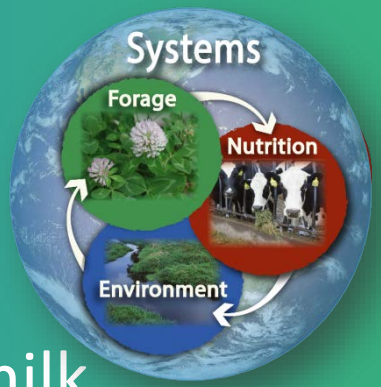


- **Farmer Benchmark:** Decreasing MUN by 1 mg/dl decreases urinary urea nitrogen excretion by 10%.
- **So what? Reducing protein feeding that reduces MUN also:**
  - Reduces farm emissions of both ammonia and nitrous oxide (most potent greenhouse gas) nitrogen by a similar amount.
  - More efficient use of feed with no loss of milk production.
  - $60 to $95/cow/year savings in protein feed costs.
  - Less environmental impact.

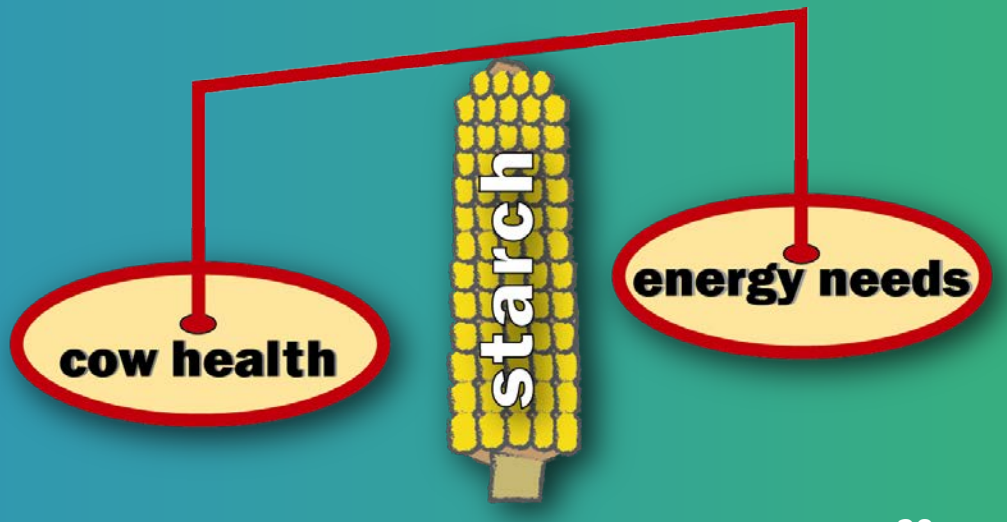
All based on a simple tool to let farmers set goals and monitor their progress!

New insights are also being developed regarding the value of organic versus inorganic N sources with significant advantages for organic sources such as manure or fixed N in alfalfa.

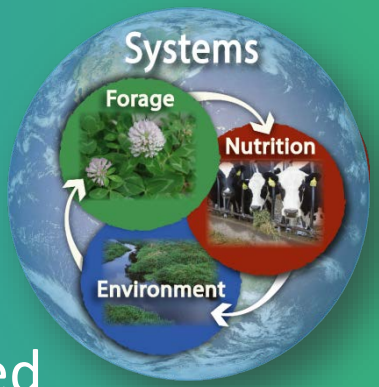
Created Tool: Starch Assay



- Starch provides essential energy to promote milk production, but too much starch can injure a cow, reduce feed efficiency, and hurt performance.
- Nutritionists & regulators needed a reliable measure for starch in animal feeds to give accurate information for diet & feed formulation.

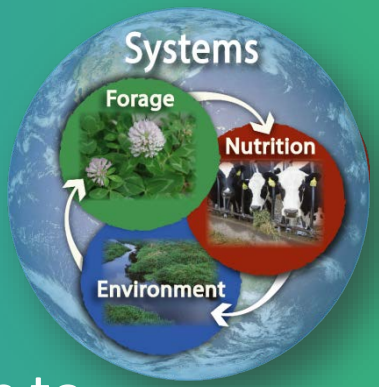


Created Tool: Starch Assay

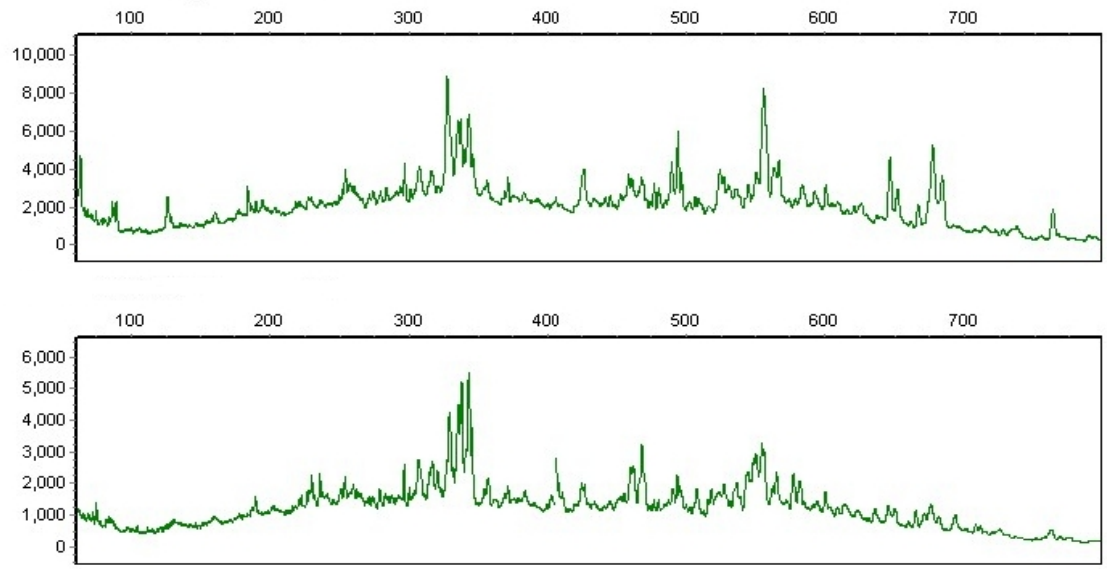


- USDFRC developed a new starch assay that can be used for diet formulation and regulatory purposes. Useful for nutritionists to make better balanced diets for cows, & for consumers to choose feeds for their animals.
- Accomplished with support from AAFCO & the AOAC Agricultural Materials Community.
- 15 commercial, research, & state laboratories collaborated.
- More than 200,000 of these assays already run per year!
- It is the new AOAC Official Method 2014.10.

Insight for the Future: Rumen Microbial Profiling



- Microbes are crucial for ruminants. They convert feeds to energy sources for the animal and are a source of protein.
- USDFRC research has determined that each cow has a unique microbial community; and the community makeup affects production and responses to diets.
- Is there a cow genetic component?
- Can the microbial profile be modified or managed for greater performance, efficiency, or health of the cow?



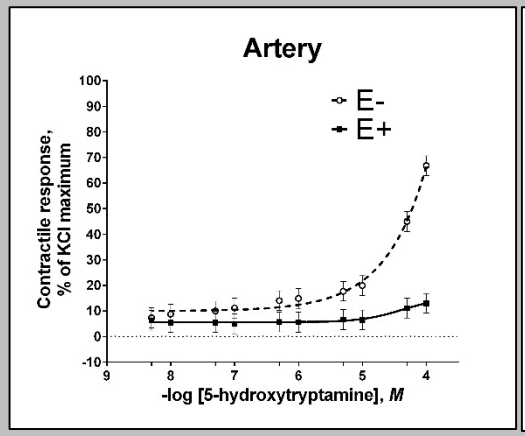
Community fingerprinting reveals that different cows vary in the amounts of different bacterial species (each peak represents a different species).

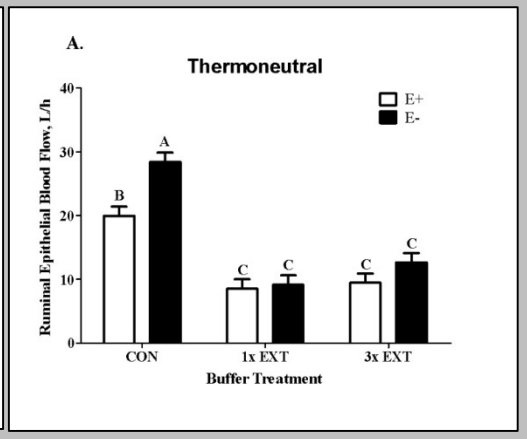
Statement of Problem: It is well documented that ergot alkaloids cause peripheral vasoconstriction. If gastrointestinal vasculature are affected similarly, then this could result in decreased nutrient efficiency and help explain observed decrease in liveweight gain also observed in animals exposed to ergot alkaloids.

Accomplishment: Demonstration of ergot alkaloid vasoactivity in ruminal and mesenteric vasculature that result in reduced nutrient absorption and may contribute to the decreases in body weight gains and production losses on toxic endophyte-infected tall fescue

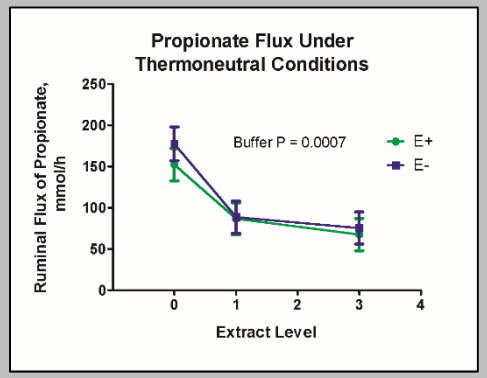
Impact: Identification of a biological mechanism by which ergot alkaloids contribute to decreased animal efficiency.

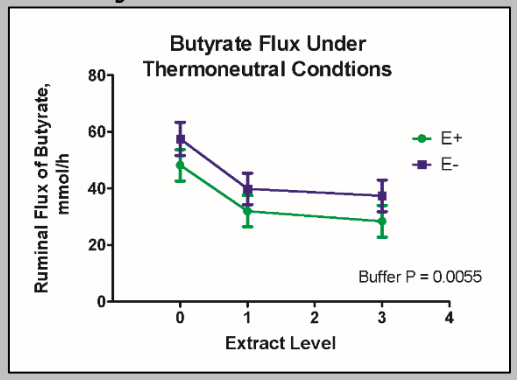
Decreased gut vasoactivity causes decreased blood flow





Decreased blood flow results in lower nutrient flux and efficiency





Statement of Problem: Management approaches are needed to mitigate the adverse effects that ergot alkaloids have on the performance and well-being of cattle that graze toxic endophyte-infected tall fescue.

Accomplishment: A 2-year grazing experiment with steers grazing toxic tall fescue pasture determined there is synergism on steer daily weight gain when combining treatments of implantation with steroid hormones and feeding soybean hulls. Feeding soybean hulls at a rate of ~0.8% of bodyweight per day was shown to mitigate fescue toxicosis.

Impact: This research identified a cost effecting method of mitigating fescue toxicosis for improving animal performance and well-being. Follow-up published research indicated that the mitigation is partly due to estrogenic activity of both implants and SBH, which can eventually lead to development of more approaches to alleviate or mitigate fescue toxicosis.

Plant-based alternatives to antibiotic growth promoters

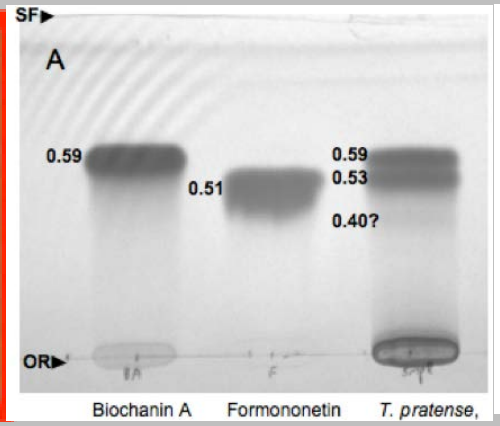
Statement of Problem: There is no replacement for antibiotic growth promoters for livestock

Accomplishment: We identified plant secondary metabolites that target the same bacteria as antibiotic growth promoters. The metabolites are: 1) biochanin A, an isoflavone from red clover, and 2) lupulone, an aromatic acid from hops.

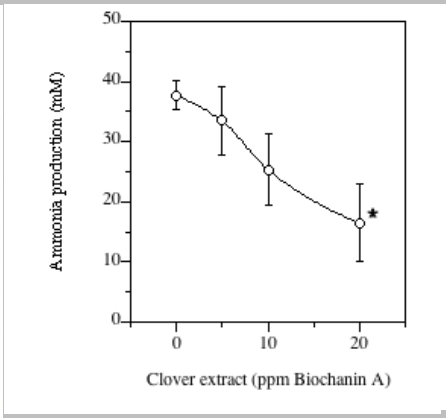
Impact: Identified plant-based alternatives to antibiotic growth promoters that can be used in backgrounding and finishing.

Key Points (Red Clover):

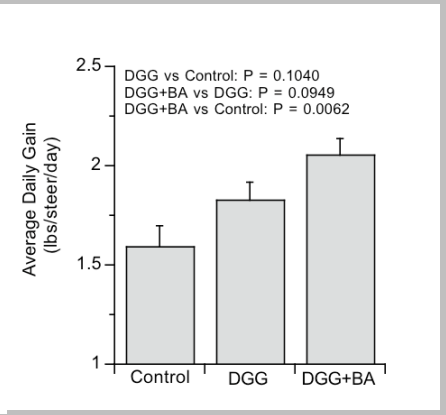
The source is a wide spread, well-accepted forage plant.



Biochanin A was identified with bioassays using the bacteria targeted by antibiotic growth promoters.



It reduces rumen conversion of valuable amino acids to ammonia, which ends up in ground water.



It promoted the growth of steers on pasture relative to controls.

Using Spatial Analysis to Compare Radiant Heater Performance

- Contract broiler growers spend approximately $500 million annually on heating fuel. Over 50 % of fuel consumed occurs during the first week when high brooding temperatures are needed. No information exists about how efficiently radiant heaters provide heat to brooding chicks.

- Combustion gas analysis, radiant flux measurements and spatial analysis were used to compare the ratio of total energy reaching the litter to energy input of the heater for six commonly used radiant heaters.

- Combustion efficiency of new brooders is approximately 80% in low pressure models compared to advertised 95%. Maximum of 41 % of energy consumed by a radiant heater reaches the litter as heat that can be used by chicks. Performance differs by mounting height and manufacturer.

- **IMPACT:** – Data show that heaters are less efficient that previously assumed and advertised. A 3% increase in brooder efficiency could potentially save $8.25 million industry-wide in the first week of brooding alone.

Radiant heater mounting assembly

Radiant flux sensor array

Evaluation Of Current Lighting Technologies For Broiler Production

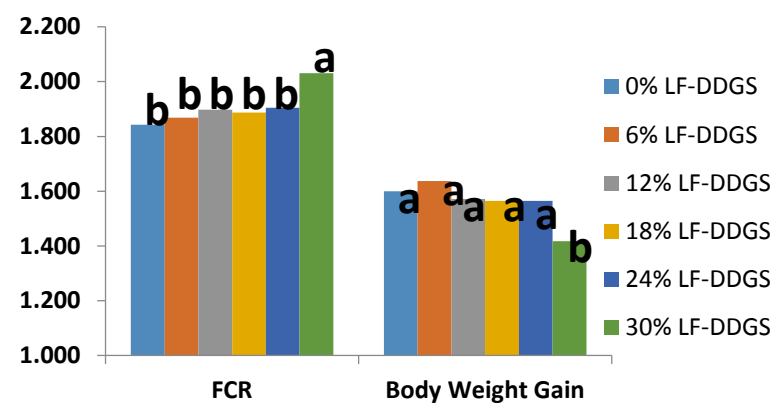
- Bird performance does not differ among lighting sources. LED lights do not improve nor impair live performance or processing characteristics
- Adjusting illuminance (intensity) according to species specific spectral sensitivity does not improve nor impair live performance or processing characteristics
- Spectral content of broad spectrum (white) light when varied among different color coordinated temperatures (Kelvin color) does not affect live performance.
- **IMPACT:** Choice of light source should be dependent upon economics of the lighting system as bulb type has not been shown to affect broiler growth.

Metabolizable Energy and Maximum Inclusion Rates of Alternative Feed Ingredients with Heavy Broilers

- Apparent metabolizable energy of low-fat DDGS (LF-DDGS) was determined in broilers at 21 and 42 d of age
  - Significantly lower AME than conventionally processed DDGS
    - Almost 56% difference in crude fat when comparing conventional DDGS vs. LF-DDGS
  - AME at 21 d: 1,148 kcal/lb vs. AME at 42 d: 931 kcal/lb
  - Significant age effect ➔ 42 d AME < 21 d AME
  - IMPACT: Energy value of LF-DDGS should be adjusted as birds age to ensure energy is not overfed and ensures production efficiency
- Inclusion rates of LF-DDGS in finishing diets for broilers (d 28 to 42)
  - Incremental levels of LF-DDGS fed in finishing diets (0 to 30% LF-DDGS inclusion)
  - Up to 24% LF-DDGS in finishing diets have no detrimental effects on live performance and production➔ Body Wt Gain and FCR significantly affected by LF-DDGS inclusion rate at 30%
  - IMPACT: Diets with 24% LF-DDGS was found to have a 12 point decrease in FCR when compared to 30% LF-DDGS. These 12 points of FCR improvement is worth $90,000/wk in feed savings

Feed conversion:
SEM 0.02591
Effect of LF-DDGS inclusion: *P*=0.0039
Linear trend: *P*=0.0099
Quadratic trend: *P*=0.1588

Body weight gain
SEM=0.04294
Effect of LF-DDGS inclusion: *P*=0.0281
Linear effect: *P*=0.0099



Determine The Interactive Effects Of Various Fat Sources And Feed Additives On Growth Potential, Meat Yields, And Blood Physiological And Biochemical Variables When Fed To Heavy Broilers

- Apparent metabolizable energy and production effiency of LF-DDGS fed with or without an exogenous carbohydrase
  - AME of broiler diets fed with 24% LF-DDGS with or without an exogenous carboyhydrase were determined at 14 and 28 d of age
    - Carbohydrase enzymes break down nonstarch polysaccharides that are unavailable to broilers for digestion. Broilers lack endogenous carbohydrase enzymes.
    - The addition of carbohydrase was found to increase AME of diets 115 and 110 kcal/g at 14 and 28 d, respectively
  - A production trial was conducted until 49 d evaluating the effects of LF-DDGS inclusion as well as optimal carbohydrase dosage
    - Diets were fed with either 0, 6, or 12% LF-DDGS and carbohydrase was supplmented at 0, 1x dose, and 2x dose
    - Diets fed with 6% LF-DDGS and supplemented with a 1x dose of carbohydrase were found to have significantly improved FCR when compared to diets fed with 0 or 12% LF-DDGS and were dependent on the inclusion rate of the carbohydrase
  - IMPACT: Energy value of LF-DDGS was increased by addition of a carbohydrase. The lowered energy value from corn oil removal can be mitigated by addition of carbohydrase.

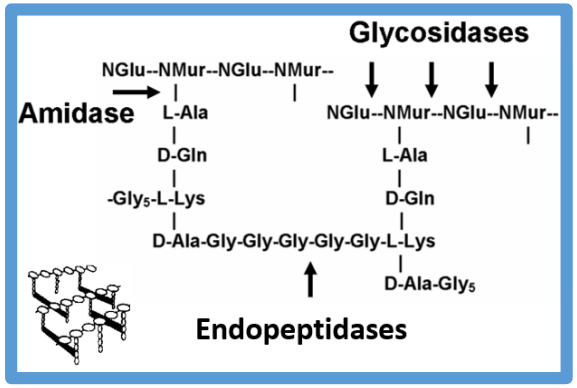
Characterize Gut Microflora And The Interactive Effects On Live Performance And Gut Microflora Of Heavy Broilers Fed Diets Containing Feed Additives And Alternative Feed Ingredients

- Effects of LF-DDGS on GIT development in male broilers reared under different weather conditions from 46 to 60 d of age
  - Diets differing in LF-DDGS inclusion (0 to 30% LF-DDGS for finisher I and 0 to 24% LF-DDGS for finisher II) were fed under two different weather conditions (summer and winter)
  - During the finisher I phase (29 to 46 d), there was no effect of LF-DDGS inclusion on GIT size or intenstinal morphology
  - During the finisher II phase (43 to 60 d), 24% LF-DDGS increased jejunal goblet cells when compared to 0% LF-DDGS diets
  - In winter conditions, 16% LF-DDGS was found to significantly decrease proventriculus, gizzard, and dudodenum length in male broilers during the finisher II phase
  - IMPACT: When feeding LF-DDGS, weather conditions were found to influence digestion of LF-DDGS and utilization of other nutrients via alteration on GIT structure

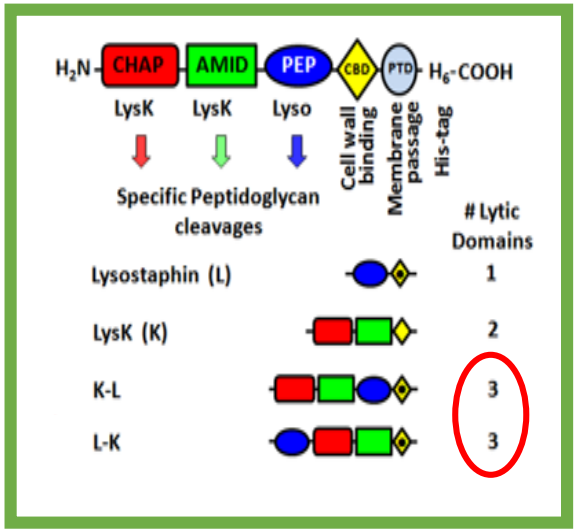
There is a need for alternatives to antibiotics:

Staphylococcal Peptidoglycan hydrolase (PGH) enzymes as Gram Positive antimicrobials.

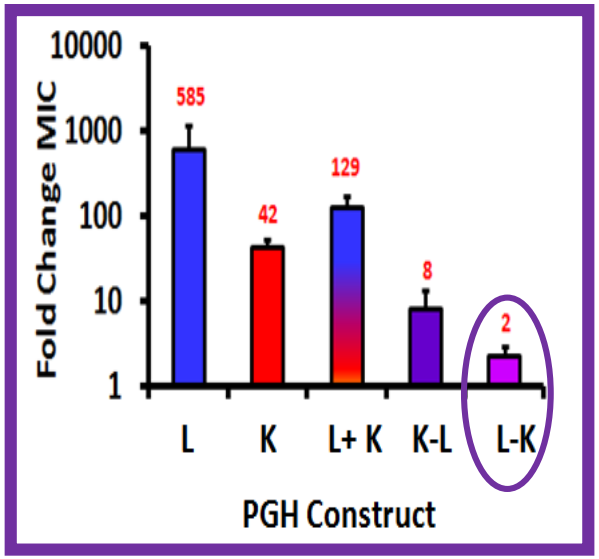
1. Multiple PGH lytic activities.



2. Fusions engineered with three unique activities.

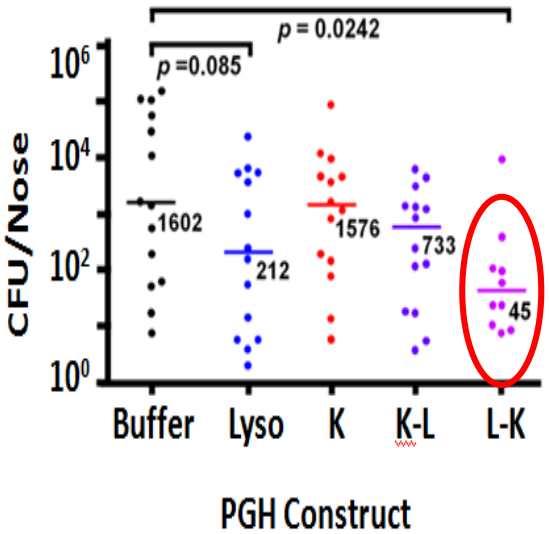


3. Triple-acting fusion are highly refractory to resistance development.



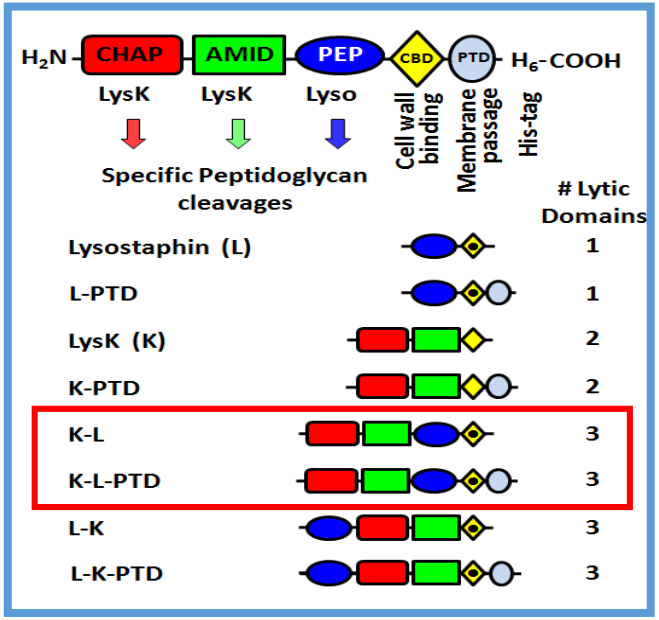
10x Repeated MIC assay. MIC does not increase.

4. Effective as mupirocin in mouse model of nasal colonization.



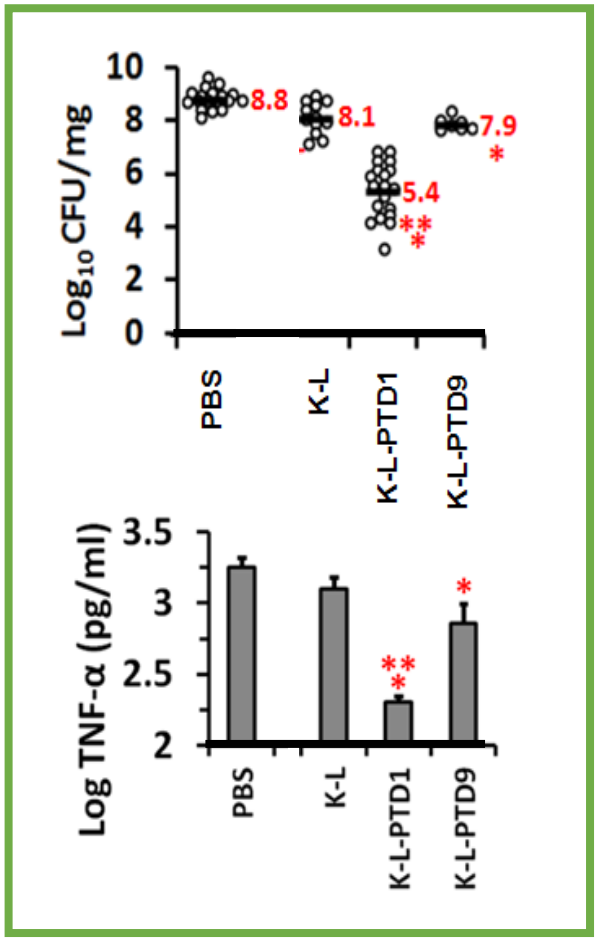
IMPACT:
Patent awarded; publicat. in submission. Potential to reduce farm to clinic resistance transmission.

PGH constructs when fused to a Protein Transduction Domain (PTD) can 1. reduce intracellular *S. aureus load, 2. reduce mammary gland colonization and 3. degrade biofilms*.



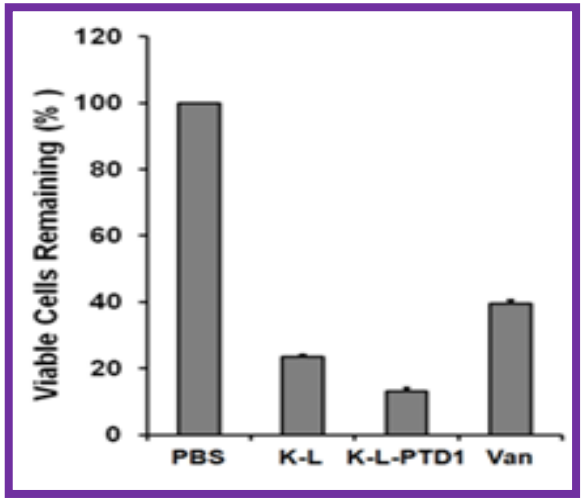
MOUSE MODEL OF MASTITIS:

reduced TNF-α (infection cytokine) and bacterial load.



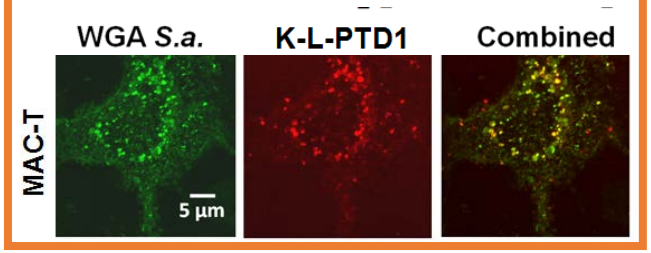
DYNAMIC BIOFILMS:

PTD harboring construct reduced dynamic biofilms.



EX VIVO CULTURED CELLS:

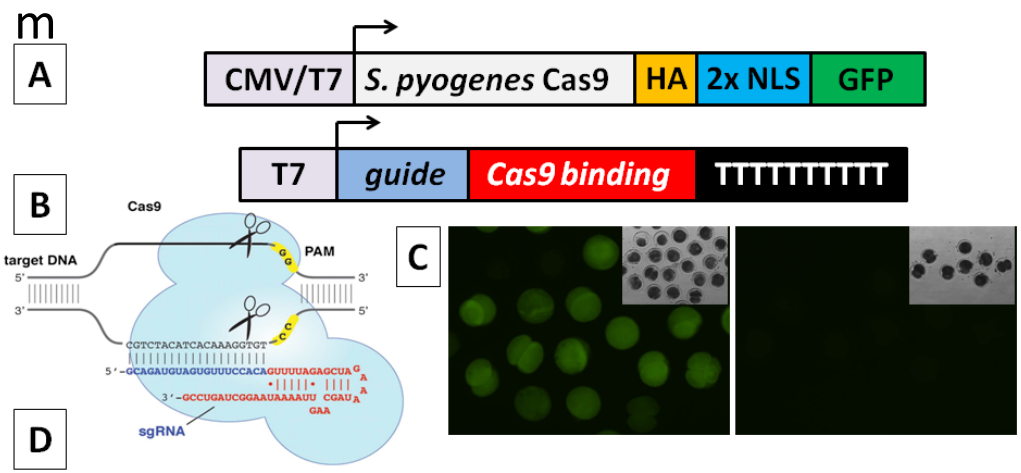
Fluorescent labeling reveals intracellular colocalization of *S. aureus* and PGH constructs.

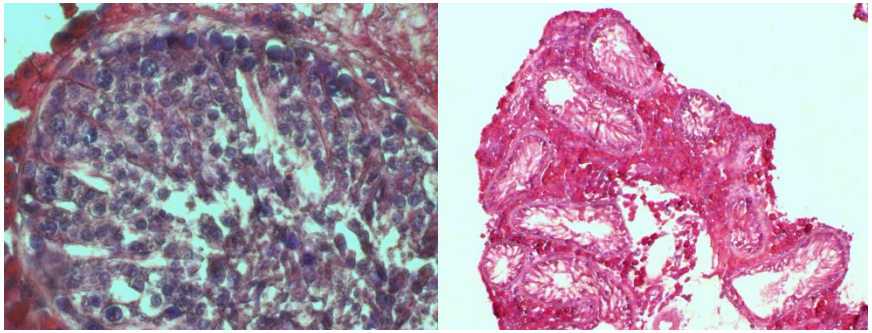


IMPACT: Patent awarded, publication submitted. Potential to reduce chronic *S. aureus* mastitis infections [and osteomyelitis in humans].

Spermatogonial stem cell (SSC) transplantation into genome edited SSC null livestock as a means to preserve valuable genetics.

- Genetic gain is achieved with selection of elite sires, but limited to lifespan of the sire.
- Transplantation of elite sire SSCs into 'SSC null' males could avoid repeated selection.
- Genome editing (CRISPR/Cas9 system): NANOS2 ablation yielded 'SSC null' males.
- The edited SSC null pigs were shipped to Wash. State Univ. for transplantation studies.





NANOS2 +/KO boars (normal spermatogenesis)

NANOS2 KO/KO boars (no SSC or spermatogenesis)

NANOS (CRISPR)

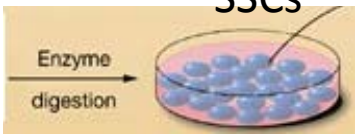
```
Nanos2   TTGGCTCCCCGGTGCCCGGGGCCTCTGGCCCTTGTCGACGATTCTGGATCAGTCCC
N1-1     TTGGCTCCCCGGTGCCCGGGGCCTCTG-CCCTTGTCGACGATTCTGGATCAGTCCC
N1-2     TTGGCTCCCCGGTGCCCGGGGCCTCTG-------TCGACGATTCTGGATCAGTCCC
N3-2     TTGGCTCCCCGGTGCCCGGGGCCTCTG-------TCGACGATTCTGGATCAGTCCC
N3-3     TTGGCTCCCGGTGCCCGGGGCCTCTGG-CCCTTGTCGACGATTCTGGATCAGTCCC
N5-2     TTGGCTCCCCGGTGCCCGGGGCCTCTG-CCCTTGTCCACAATTCTGGATCATTCCC
N5-3     TTGGCTCCCCGGTGCCCGGGGCCTCTG-------TCGACGATTCTGGATCAGTCCC
N6-1     TTGGCTCCCCGGTGCCCGGTGCC-CGGGCCCTTGTCGACGATTCTGGATCAGTCCC
N7-2     TTGGCTCCCCGGTGCCCGGGGC-------CCTTGTCGACGATTCTGGATCAGTCCC
N7-3     TTGGCTCCCCGGTGCCCGGGGCCTCTG--CCTTGTCGACGATTCTGGATCAGTCCC
N10-2    TTGGCTCCCCGGTGCCCGGGGCCTCT-------GTCGACGATTCTGGATCAGTCCC
N11-2    TTGGCTCCCCGGTGCCCGGGGCC------CCTTGTCGACGATTCTGGATCAGTCCC
N12-2    TTGGCTCCCCGGTGCCCGGGGCCTCTGC-CCTTGTCGTCAATTCTGGATCAGTCCC
N12-3     TTGGCTCCCCGGTGCCCGGGGCCTCTG-------TCGACAATTCTGGATCAGTCCC
```

100% edited progeny

Future directions

Elite Testes

SSCs



Isolate and inject SSCs

IMPACT: Awaiting transplantation studies at Wash. State Univ.

Improving the Reproductive Performance of Male Turkeys

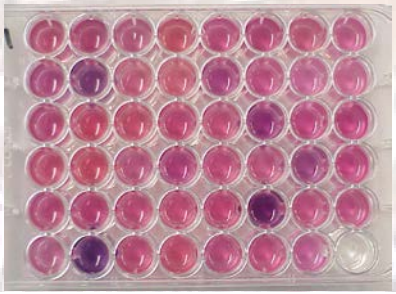
Problem: The turkey industry relies exclusively on artificial insemination, a time and labor-intensive process, to reproduce birds in commercial operations. With freshly collected semen, fertility rates are typically 94-98%; however, if semen is held for longer than 6 hours prior to insemination, fertility rates drop to 40%. These low fertility rates are not economically acceptable.

Accomplishment: New semen extender supplements improved the fertility of turkey semen stored for 24 hours prior to insemination.

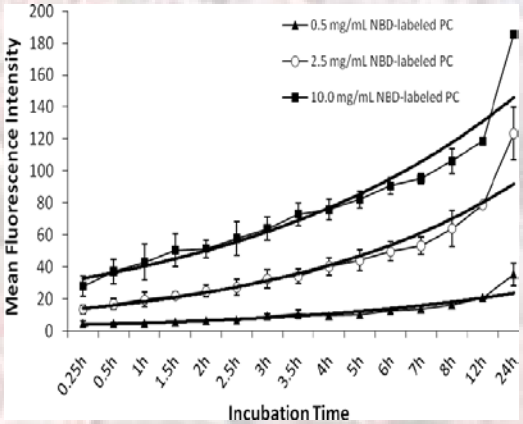
ARS scientists have been evaluating the biological basis for the drop in fertility associated with stored turkey semen, and have shown that the (1) sugar residues on the surface of the sperm membrane change and (2) lipids within the sperm membrane are degraded when semen is held for longer than 6 hours at cold temperatures.

Systematic evaluation of exogenous sugars and lipids established that turkey sperm exhibit dose- and time-response mechanisms for uptake during hypothermic semen storage, counteracting the effects of lipid peroxidation and carbohydrate loss that impair sperm function.

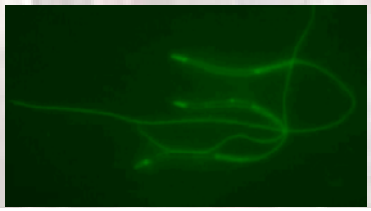
Impact: Use of the new semen extender improved the fertility rates of semen stored for 24 hours from *40 to 85%.* This represents a significant advance in poultry semen storage technology that will save producers time and money when reproducing flocks.



Colormetric assay reflects the degree of sperm lipid damage.



Phosphotidylcholine (PC) uptake by turkey sperm.



Sperm uptake of labeled sialic acid.

Enabling Genomic-based Improvement Programs for the Turkey Industry

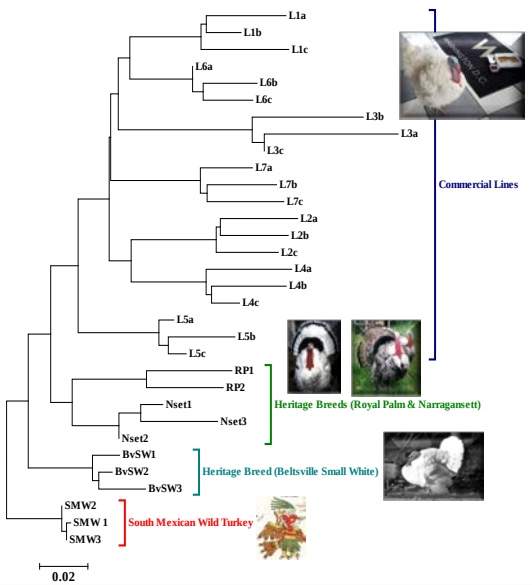
Problem: Genetic improvement for turkeys has been achieved using selective breeding programs based on physically-measureable traits. For less measurable traits such as immunity and reproduction, more sophisticated genomic selection is needed. While genomic resources are available for chicken production, these tools have not been available to the turkey industry.

Accomplishments: Multi-platform next generation sequencing of the domestic turkey genome and development of a new genetic tool (SNP array) for turkey producers.

ARS scientists, in partnership with VPI and UMD, sequenced and annotated the genome of the domestic turkey using a unique sequencing strategy of data from both Roche 454 and Illumina GAII platforms.

ARS scientists conducted a broad assessment of 11 different turkey lines and breeds and discovered 5.49 million SNPs that are potential markers for genetic improvement.

Impact: A comparative analysis of the turkey, chicken and zebra finch genomes supports the characteristic stability of avian genomes and identified genes unique to each species and the avian lineage. An array containing *60,000 of the best genetic markers was made publically available* for turkey producers and researchers.



Phylogenetic tree of 7 commercial turkey lines and 4 turkey breeds, including the ancestral South Mexican wild turkey.

Domestication of the commercial turkey: a true U.S. immigrant.

Improving Hatchability of Broiler Eggs

Problem
In 2013, 10 billion broiler eggs and 340 million turkey eggs were incubated: only 83% and 79% hatched, respectively. For logistical reasons, storing eggs prior to incubation is a common practice. However, when stored at 18C° 7 days or longer, there is a progressive increase in early embryonic mortality during incubation. An increase of just 1% in broiler egg hatchability would significantly increase the number of broiler hatching eggs.

Accomplishments
Confirmed previous work that short pre-incubations (PI) during egg storage increase embryo survival (table in upper left) by increasing the developmental stage of the embryo prior to incubation (figure lower right).

Indentified cellular and molecular alterations to the blastoderm and blastodermal cells as a result of egg storage with and without PI before and after incubation.

Discovered that embryos in fresh laid turkey eggs are less developed than those described for the turkey embryo in 1993: subsequently revised the turkey embryo staging table.

Improved methods to isolate embryo and stage embryonic development that have been successfully applied to chicken, turkey, quail, duck, and goose embryos.

Relative expression of genes associated with oxidative stress, apoptosis, and fatty acid metabolism compared in fresh laid eggs and eggs stored for 21 days with and without PI.

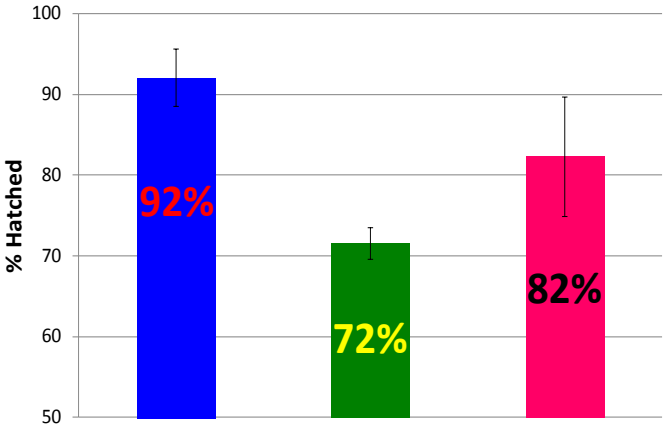
Impact
Pre-incubating eggs prior to incubation to reduce embryo mortality is rapidly being adapted by the poultry industry.

Contributed to the development of the first commercial incubator designed specifically for PI.

Commercial hatchery personnel and poultry scientists utilizing methods in hatcheries and laboratories.

As a result of this project ARS scientist is recognized as international expert in hen egg early embryonic development and impact of egg storage on hatchability.

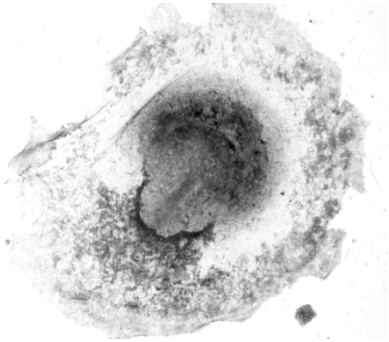
Hatch of Stored Broiler Eggs w/ and w/o Pre-incubations (PI)



Data from 3 experiments: BLUE, eggs stored 4 d; GREEN, eggs stored 21 d, no PI; PINK, eggs stored 21 d, with PI

Pre-incubation (PI) of stored eggs advances embryonic development resulting in a significant decrease in embryonic mortality

Blastoderm at time egg laid: one cell layer



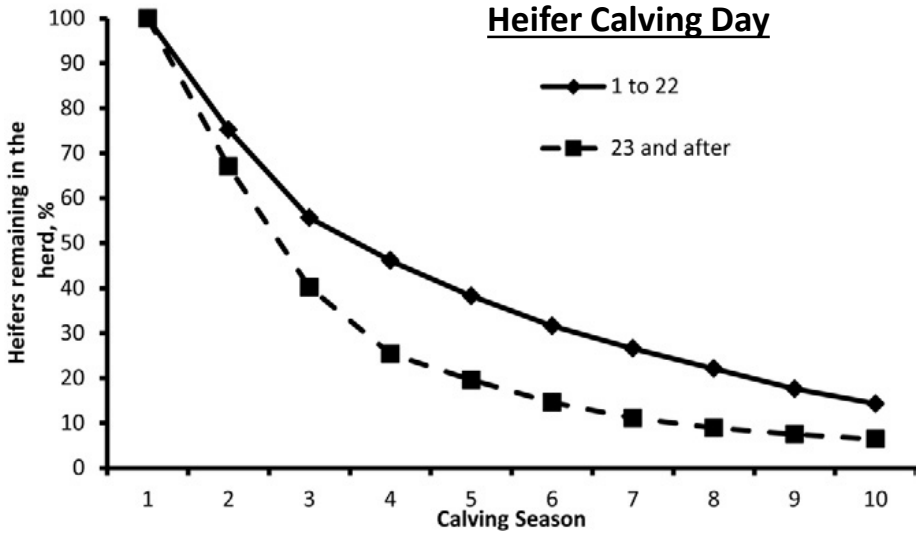
After PI, embryo is forming third cell layer

The Problem: Weak return on investment for replacement heifers

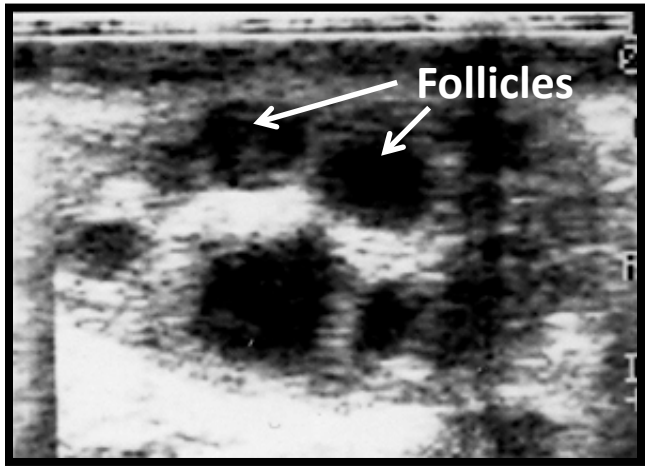
A replacement heifer must wean between 3 and 5 calves to pay her development costs

A survey of cow-calf producers indicated that only 50% of replacement heifers produce 3 calves

Heifers that give birth early stay in the herd longer



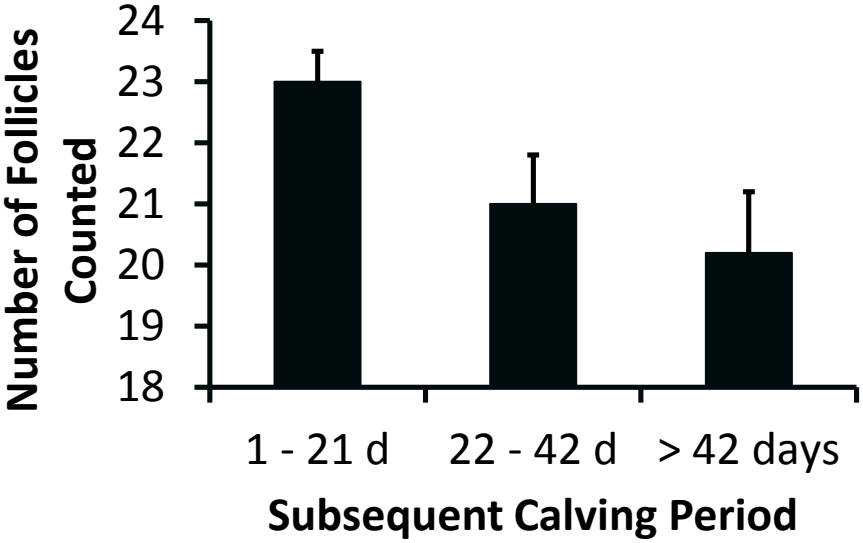
We can select heifers that have increased likelihood of remaining in the herd



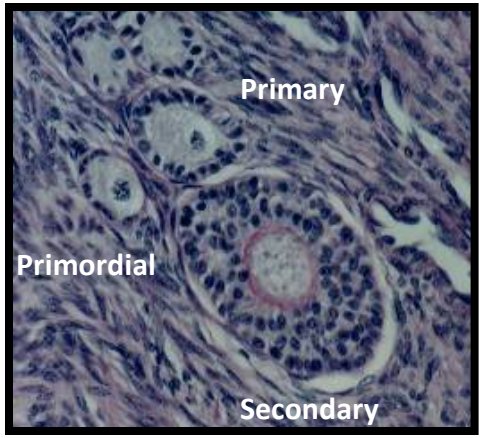
Bovine Ovary

Ultrasonographic examination of the ovaries before breeding to determine follicle number

Heifers with more follicles conceive earlier in their first breeding season



The way we feed a heifer after weaning can change her ovarian phenotype

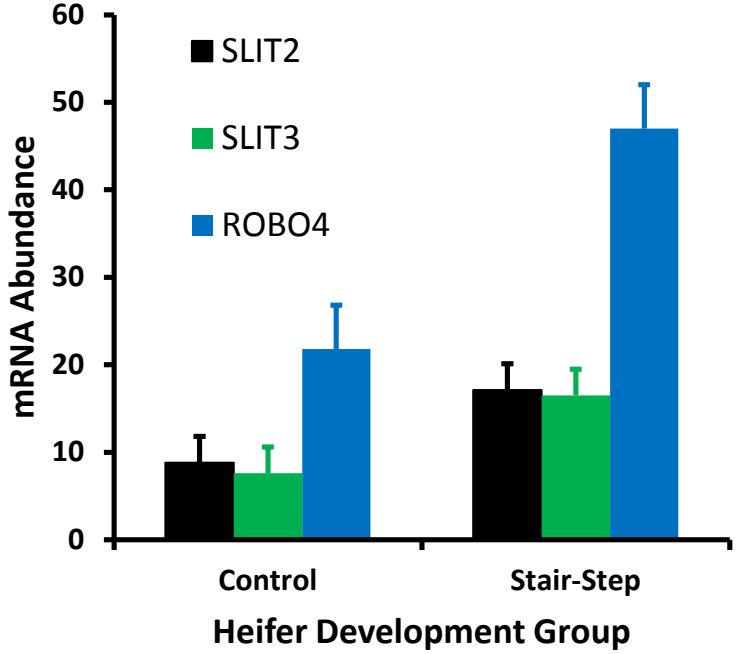


Developing heifers using a stair-step protocol increased the number of microscopic follicles in their ovaries

		Preantral Follicle Stage		
Diet	**n**	**Primordial**	**Primary**	**Secondary**
Control	**6**	**52.4 ± 14.0**	**22.2 ± 4.6**	**3.9 ± 0.8**
Stair-Step	**6**	**112.5 ± 14.0**	**25.8 ± 4.6**	**5.6 ± 0.8**
***P*-value**		**0.004**	**0.58**	**0.13**

Nutritional programming of gene function in the bovine ovary

- To investigate epigenetic mechanisms, RNA-sequencing identified 291 differentially expressed genes
- Transcript abundance of genes involved in follicle formation and growth was greater in the ovaries of stair-step heifers



Impacts

- Veterinarians are adopting follicle counts into pre-breeding exams
- We are supplying the basic knowledge required to apply nutritional programming on the farm
- Nutritional programming decreases the development costs of a heifer while improving her ovarian phenotype to increase her reproductive longevity

Problem: Capacity to satisfy protein food needs of an expanding global population will require greater food production on an ever decreasing availability of land available for agriculture.

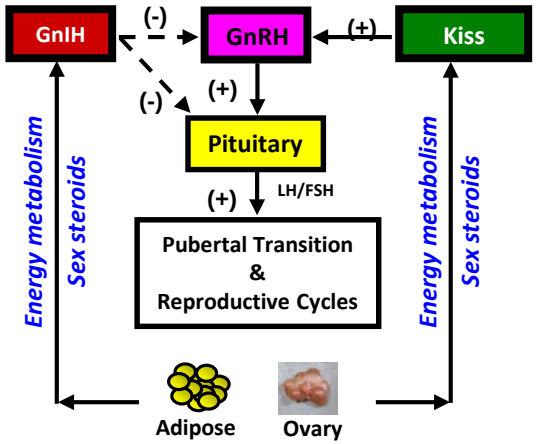
Possible solution: The ability of ruminant livestock to convert forages and water of insufficient quality to contribute to human dietary needs to high quality sources of protein and other nutrients for human nutrition.

More efficient practices and technologies enhancing sustainability of ruminant livestock production will provide critical capacity to meeting dietary requirements of the growing world population

Gilt Development and Sow Longevity

Problem: breeding herd retention of gilts and sows

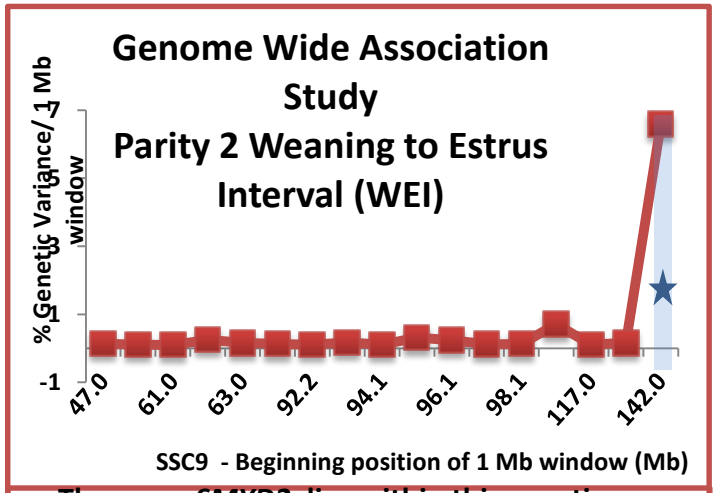
Pubertal Development



- Defined a hormonal system that inhibits secretion of reproductive hormones.
- Discovery was used by scientists to explain the biological basis of genetic markers identified through genomic studies on pubertal development.

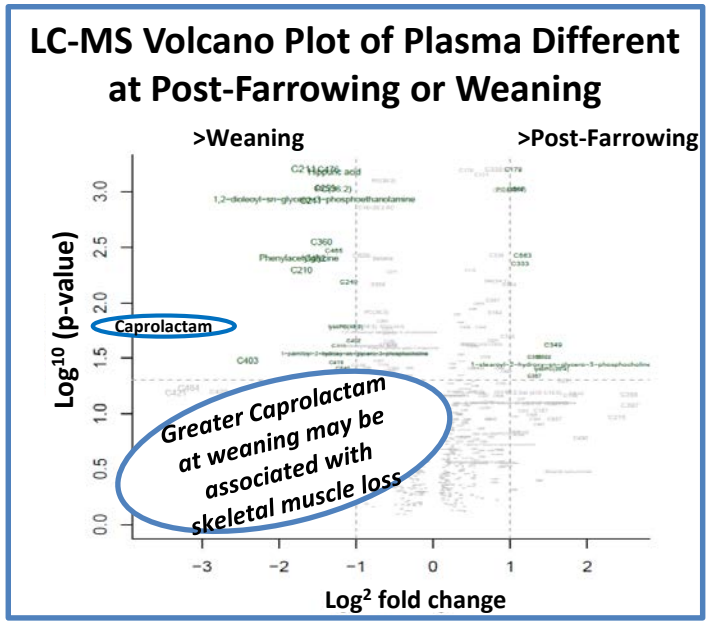
Dietary management of gilts

- Increased preweaning growth rate correlated with reduced age at puberty
- Collaborated with university and industry partners to develop a diet that slows growth of gilts.
- Diet is being used on a commercial farm, in a study funded by the National Pork Board, to test effect of reduced growth rate on retention of gilts in the herd.



★ The gene, *SMYD2*, lies within this genetic region.

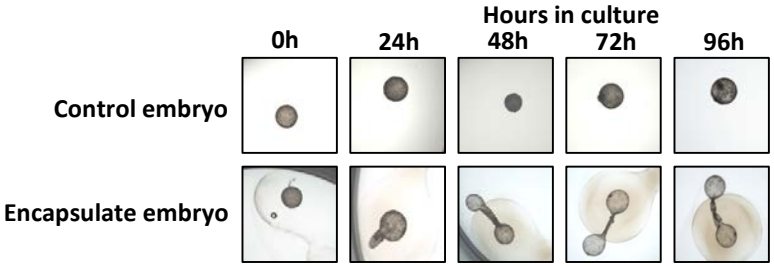
- SMYD2 inhibits Estrogen Receptor 1, a key player in reproductive activity.



Pregnancy and the Neonatal period

Problem: Reduced litter size and increased preweaning mortality

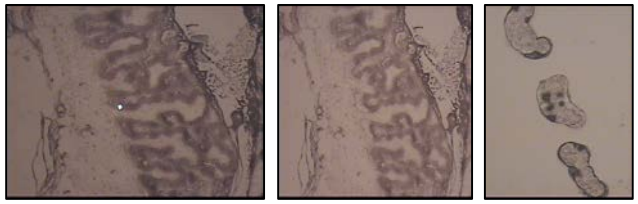
Development of a culture system using alginate hydrogel encapsulation as a 3D matrix to study the initiation of elongation



- This system encapsulates embryos, maintains viability and architecture, and facilitates morphological changes.
- Used to identify and characterize specific factors that regulate embryo elongation in real-time and in a control environment.
- Identify mechanisms to normalize elongation between littermates to reduce within-litter birth weight variability and improve piglet survival.

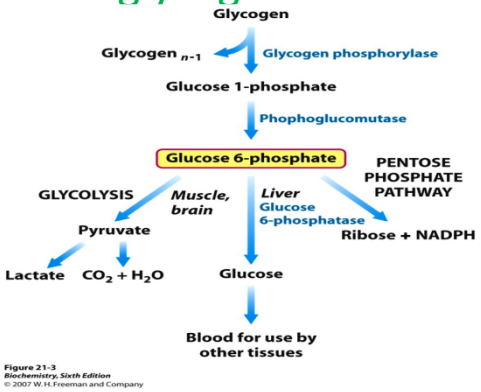
Evaluation of transcriptional differences between specific cell types (tall columnar & short cuboidal) within the chorionic villi

Laser capture of the placenta to isolate specific cell types



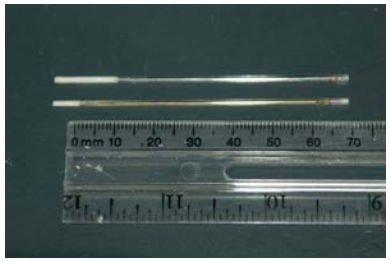
- Differential gene expression using Illumina sequencing indicated that placental fold development, which controls the maternal/fetal interacting surface, was primarily controlled by short cuboidal cells
- Identified unique patterns of expression for various genes that produce nutrient specific transporter proteins between these cell types. Most nutrient transport appears to occur via short cuboidal cells. Unexpected results included high expression of insulin dependent glucose transporters and proton dependent inositol transport

Neonatal glycogen metabolism



- Improved glycogen turnover linked to survival

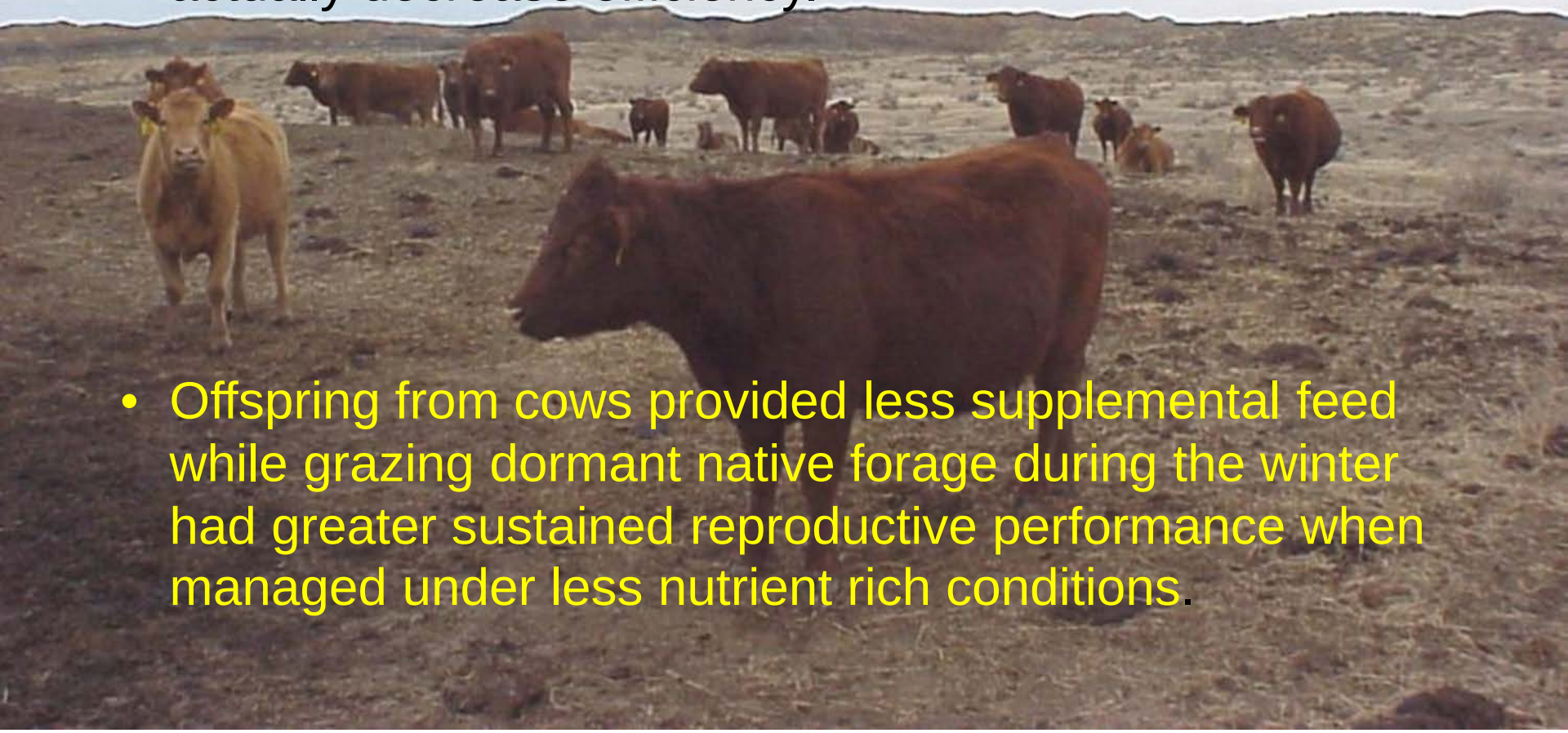
Colostrum acquisition by piglets



- Invented the immunoglobulin immunocrit procedure
- Identifies piglets that do not get colostrum and are at high risk of mortality- colostrum management
- Immunocrit linked to adult fertility

Research conducted over the last 14 years has focused on identifying environmental, management and genetic factors affecting **lifetime** production efficiency in beef cattle.

- Results provide evidence that industry recommendations for beef herd managements may be excessive and actually decrease efficiency.

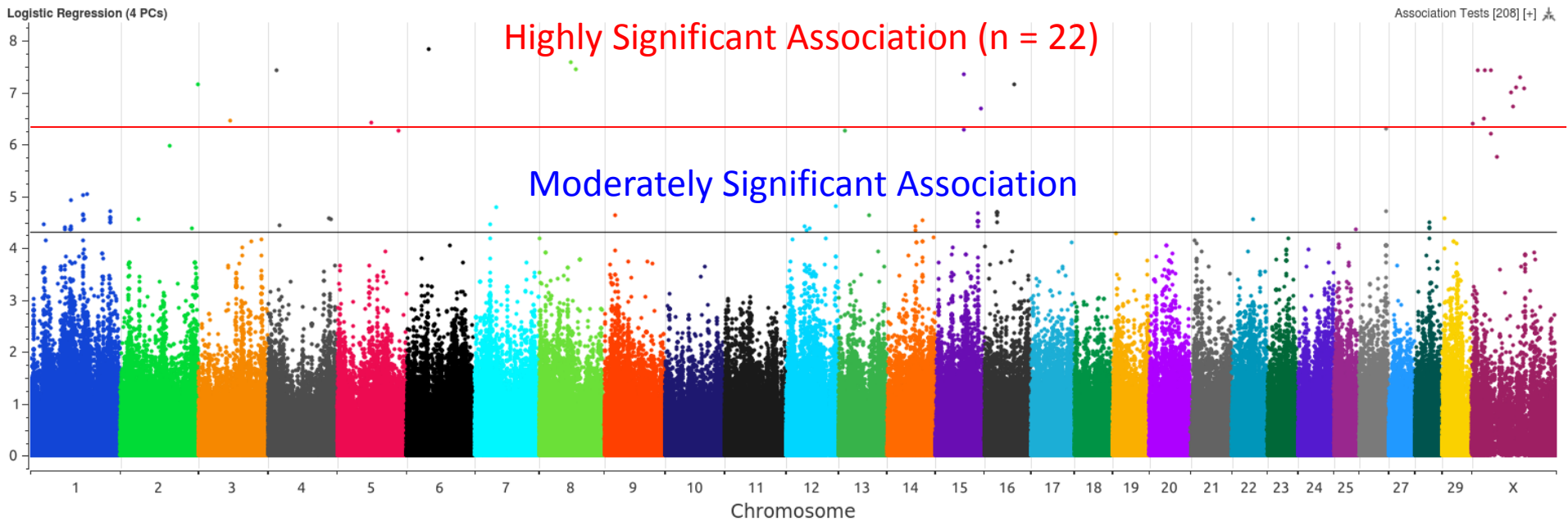


- Offspring from cows provided less supplemental feed while grazing dormant native forage during the winter had greater sustained reproductive performance when managed under less nutrient rich conditions.

Strategic feeding of replacement heifers developed on native range resulted in $80 savings per animal compared to conventional approach of feeding harvested feed in a feedlot

Equivalent to ~ $360 million/yr annual saving in U.S, and decreased inputs from tillable land

Functional genomic and genomic approaches have identified 41 gene products and 22 chromosomal regions associated with reproductive function.



High Fertile = 30 heifers with 100% pregnancy rate to ET

Subfertile = 21 infertile heifers (0% ET PR)
+ 12 subfertile (25% ET PR and ≤ 25% AI PR)
33 heifers

Cattle Temperament Effects Sickness Behavior

The below video clips (click on image for video menu) document the diverse sickness behaviors observed in calm versus temperamental cattle. Note the increased respiration rate and lethargic behavior of the calm calf compared to the agitated, alert behavior of the temperamental calf. Typically, the behavior of temperamental cattle will change very little or not at all after an immune challenge. At times, temperamental cattle may even become more agitated or excited following an immune challenge.

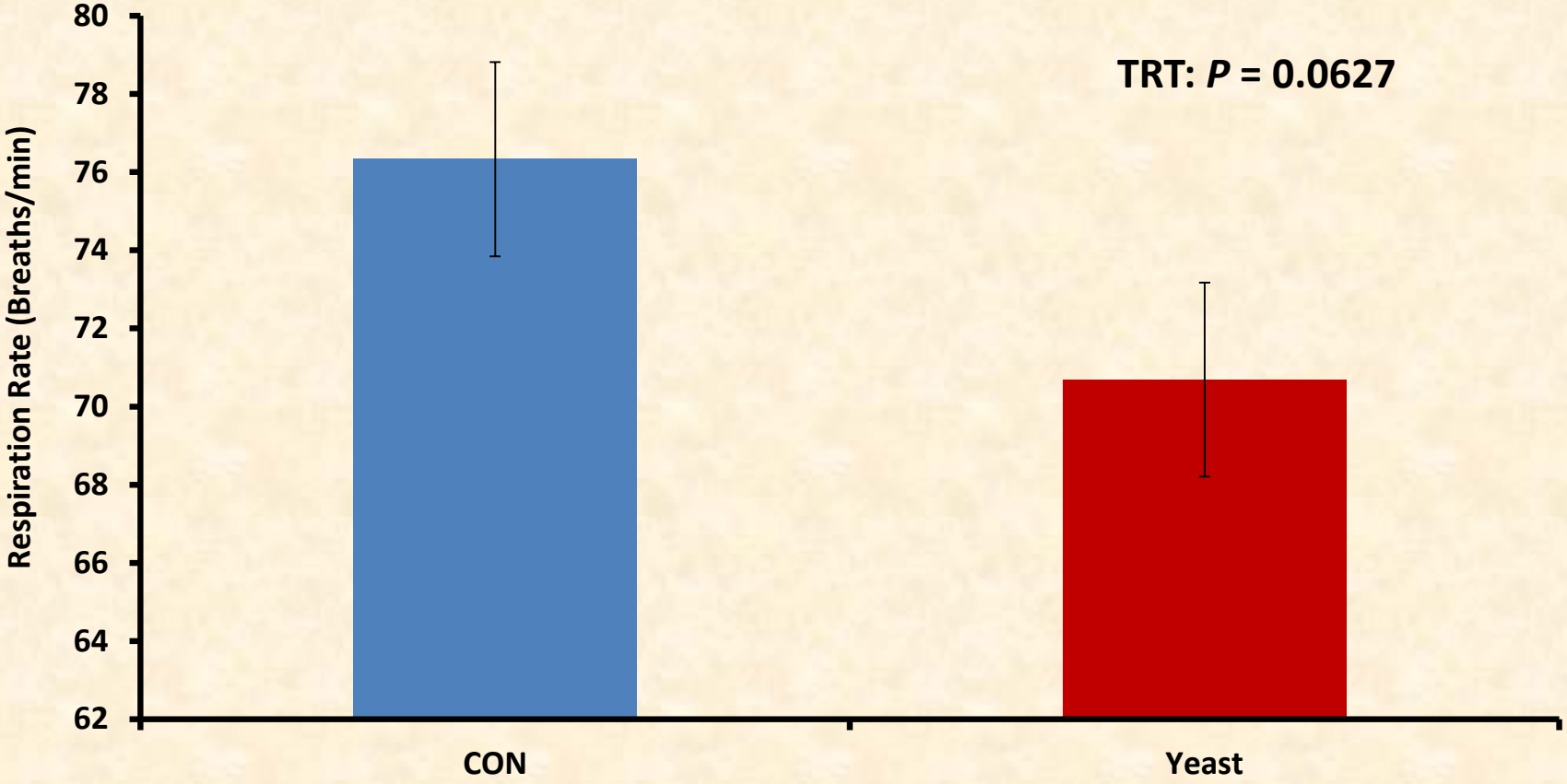
Calm

Temperamental

USDA-Agriculture Research Service
Livestock Issues Research Unit
Lubbock, Texas

Yeast Supplementation Reduces Effects of Heat Stress

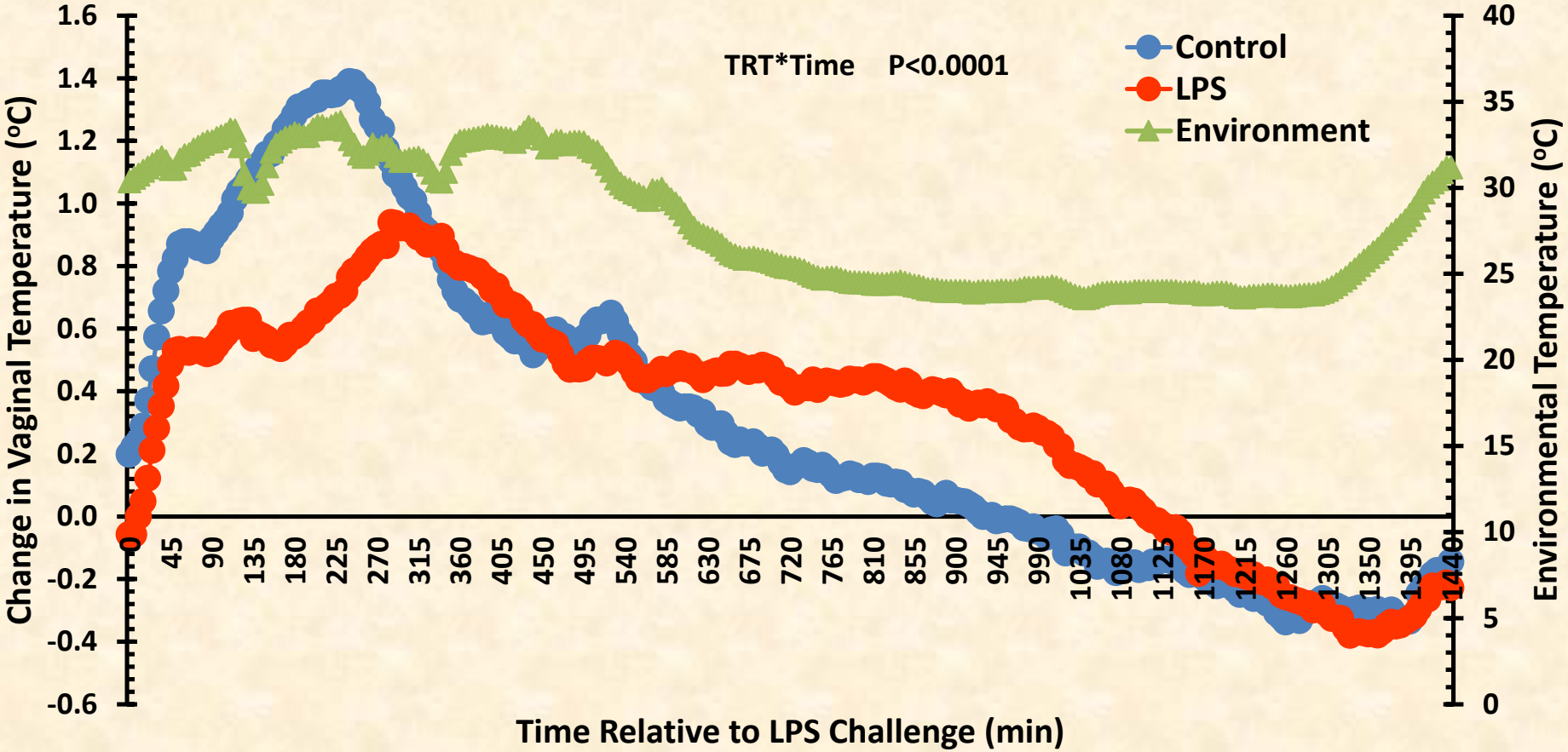
Scientists within the Livestock Issues Research Unit have conducted a series of studies with industry collaborators that have demonstrated that feeding a yeast supplement to cattle reduces the detrimental effects of immune challenges and heat stress. With regard to heat stress, results have demonstrated that yeast supplementation reduces body temperature and respiration rates, while increasing water intake. The below figure represents the respiration rates of feedlot heifers that were fed a standard feedlot ration with (Yeast) or without (CON) a yeast supplement for 50 days and then exposed to a controlled heat stress.



USDA-Agriculture Research Service
Livestock Issues Research Unit
Lubbock, Texas

Prenatal Programming of the Immune System in Cattle

Scientists within the Livestock Issues Research Unit have conducted a series of studies in swine and cattle with university collaborators that have demonstrated that applying various treatments to the dam during gestation can alter the stress and immune responses of their offspring. The below figure represents data from one study demonstrating that administering E. coli derived endotoxin (i.e., lipopolysaccharide; LPS) to gestating cows during the 3rd trimester reduced the febrile response in their heifer calves when exposed to LPS at 6 months of age.



Beak Trimming: Hot Blade vs. Infrared

- More than 336 million hens, to reduce cannibalism.
- ARS has shown that traditional hot-blade trimming causes both acute and chronic pain, thus creating a welfare problem.

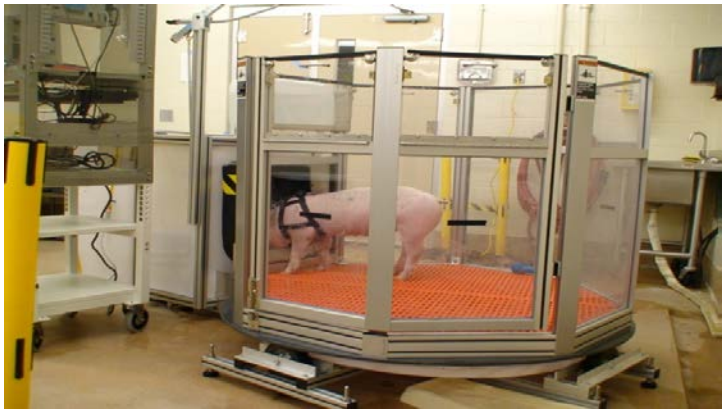
- Our research found that infra-red beak trimming is less painful.
- The industry has readily adopted this practice as well as European countries with some abolishing hot-blade trimming in favor of infra-red trimming.

The chicks on the left have been hot-blade trimmed, note the squinted eyes and posture.

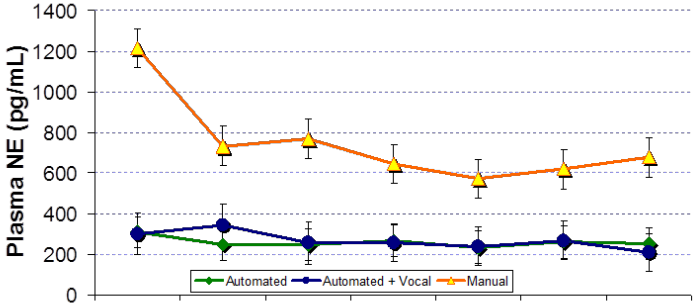
Chicks on the right have been infra-red trimmed, note feeding behavior.

Non-Invasive Measurement of Stress

- Research on stress must measure the stressful state of animals, yet, the act of taking biological samples can cause a stress response and thus lead to erroneous conclusions.
- To address this conundrum ARS partnered with a High Tech Life Sciences corporation to develop a novel housing system (Development of PigTurn; Culex-L®) for stress-free simultaneous collection of behavior, biofluids and electro-cardiac activity.
- This resulted in patented device and the establishment of a core facility (Purdue Translational Pharmacology Facility) on Purdue University's Discovery Park
- The system is currently being used by drug companies for in vivo testing of New Chemical Entities to treat human disease; and by ARS scientist to discover true stress reactions of swine.



Plasma norepinephrine response of pigs restrained by a person or when taken by the PigTurn machine.



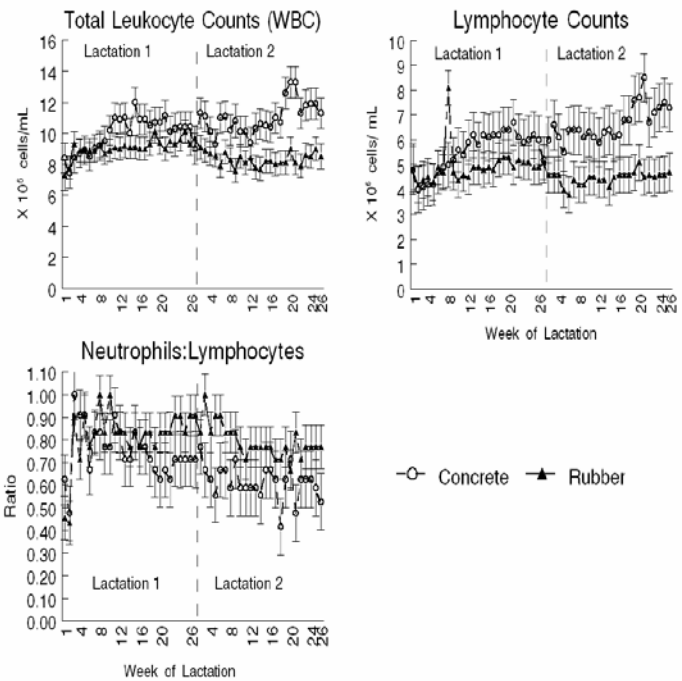
The blue line are data from pigs which are sampled from the machine but can hear a neighbor pig being restrained.

Rubber Floors for Dairy Cows

The incidence of lameness in dairy cows plagues the industry; decreasing farm revenue and cow welfare.

ARS found that dairy cows housed with rubber mats in front of the feed bunk had less incidence of lameness, less interleukin-1 beta, a measure of inflammation. In addition, cows on concrete had elevated leukocytes, a classic indication of chronic pain.

This research shows that by providing better floor surfaces for cows, lameness can be reduced. Further, it shows that housing cows on concrete floors keeps cows in a chronic state of inflammation and possibly some level of chronic pain.



Eicher et al., 2013; J. Dairy Sci.

Selected as Featured Article. J. Dairy Sci, 2013; and 1 of top 10 papers of 2013 that "***increase our understanding of the pathophysiology of disease or change the way we diagnose, treat, and control disease of food animals***" by the American College of Veterinary Internal Medicine

Questions on Component 1?

Break

Agenda *continued*

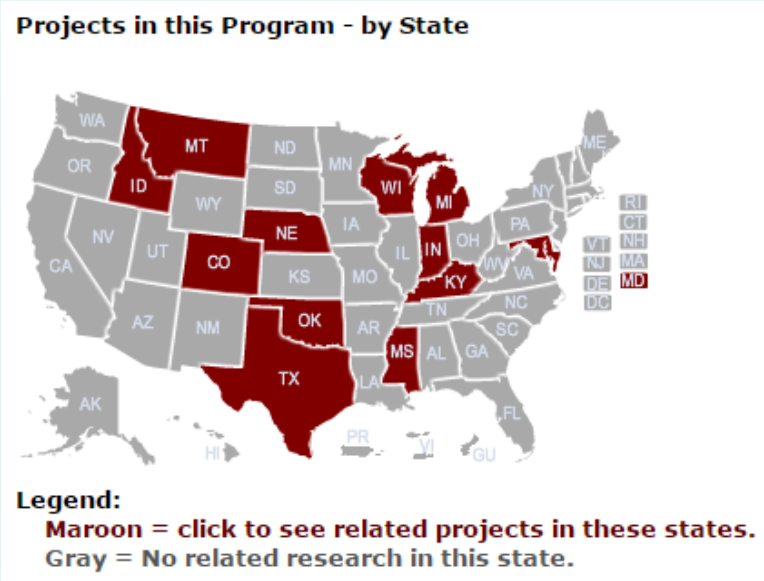
- Program Summary Information
  - Caird Rexroad
- Accomplishment Stories: Components 2 and 3
  - ARS Scientists
- Panel Discussion (Panel Only)
- Panel Report and Debrief (Panel, ONP)

Program Information

Personnel and Locations

Fiscal Year	Number Scientists	Number Locations	Number Research Projects
2010	99	18	32
2011	91	17	32
2012	96	10	29
2013	84	11	28
2014	83	13	29

- $48 Million/year
- $53 Million/year w/Extramural
- Funding Sources 2009 - 2014
  - University 22
  - Government 31
  - Industry 55
  - International 11



Program Information

Technology Transfer, Collaborations, and Publications*

Fiscal Year	Applications for New Patents	Active CRADAs	MTRAs	Refereed Journal Articles
2010	2	6	5	160
2011	1	5	9	150
2012	5	3	5	150
2013	1	4	5	170
2014	1	3	17	135

*Scientists maintain or collaborate on 11 databases.

Mentoring and Editorship

	Postdoctoral Fellows	Graduate Students	Undergraduate Students	Visiting Scientists	Editorships
Total FY2010–FY2014	73	68	143	197	48

International Collaborations

COUNTRY	TOTAL	COUNTRY	TOTAL
ARGENTINA	2	NETHERLANDS	21
AUSTRALIA	36	NETHERLANDS ANTILLES	1
AUSTRIA	16	NEW ZEALAND	12
BELGIUM	7	NIGERIA	3
BRAZIL	59	NORTHERN IRELAND	1
CANADA	56	NORWAY	10
CHILE	1	PAKISTAN	8
CHINA	14	PHILIPPINES	1
CZECH REP	1	POLAND	1
DENMARK	11	REPUBLIC OF IRELAND	4
EGYPT	3	ROMANIA	2
FINLAND	2	RUSSIA	9
FRANCE	25	SAUDI ARABIA	3
GERMANY	11	SCOTLAND	13
GHANA	1	SLOVENIA	1
GRENADA	1	SOUTH AFRICA	4
INDIA	8	SPAIN	14
IRAQ	2	SWEDEN	12
ISRAEL	9	SWITZERLAND	15
ITALY	43	THAILAND	1
KAZAKHSTAN	3	TUNISIA	6
KENYA	12	TURKEY	5
NORTH KOREA	1	UGANDA	3
MALAWI	1	UNITED KINGDOM	41
MEXICO	12	URUGUAY	2
		VENEZUELA	1
531 collaborations in 52 countries			

Component 2: Genetic Improvement - Understanding, Improving, and Effectively Using Animal Genetic and Genomic Resources

- Problem Statement 2A: Develop bioinformatic and quantitative genomic capacity and infrastructure for research in genomics and metagenomics.
- Problem Statement 2B: Identify Functional Genomic Pathways and Their Interactions.
- Problem Statement 2C: Preserve and Curate Food Animal Genetic Resources.
- Problem Statement 2D: Develop and Implement Genome-Enabled Genetic Improvement Programs.
- Problem Statement 2E: Improved Techniques For Genetic Modification and Genetic Engineering of Food Animals.

Two partner projects to develop tools and knowledge

Long-term goals :

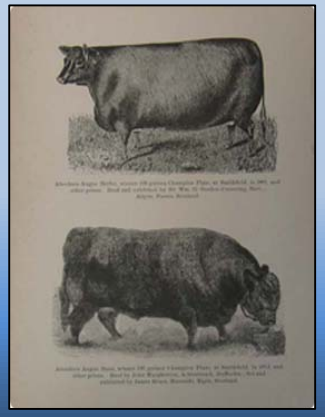
Develop bovine genomic and genetic resources and tools

Bring these tools to bear on issues of sustainability, adaptation, and animal welfare

Develop knowledge and tools for evaluating the role of microbial populations associated with the animals and their production environment

Genetic improvement of beef cattle : more beef with fewer animals

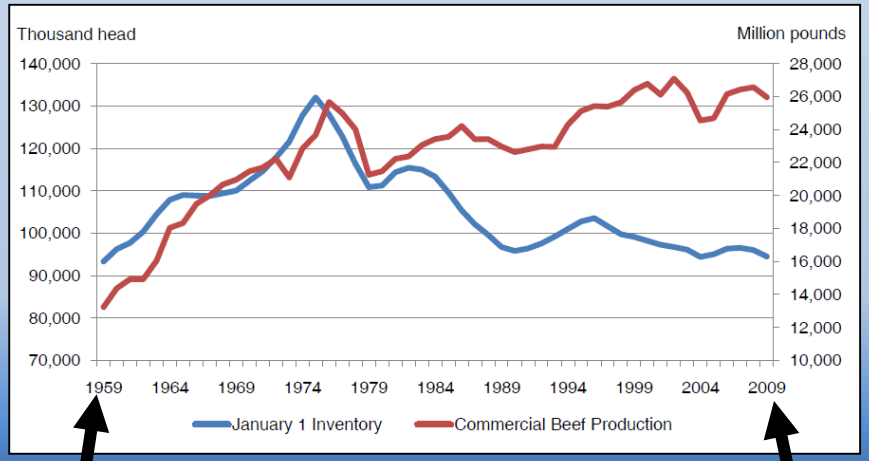
- Genetic selection accounts for much of increased beef production (estimated 1/3) with fewer animals



Drawings of cattle 200 years ago

A modern beef animal

- U.S. cattle herd size / amount of beef produced



1969

USDA, NASS, 2010

2009

Using genetics and genomics to address stakeholder needs

- **Industry concerns**
  - Feed cost, disease, reproduction (performance)
  - Environmental impact, welfare, food safety (social concerns)

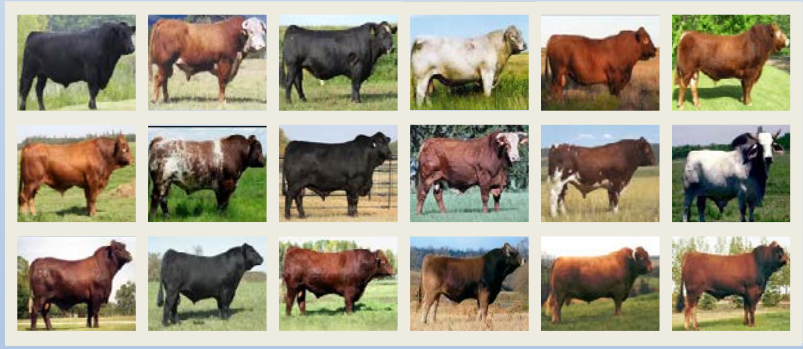
The USMARC "2000 bull project"

Genotyping/sequencing the most important industry bulls

Developing genetic markers with predictive merit for meat quality (tenderness, leanness)

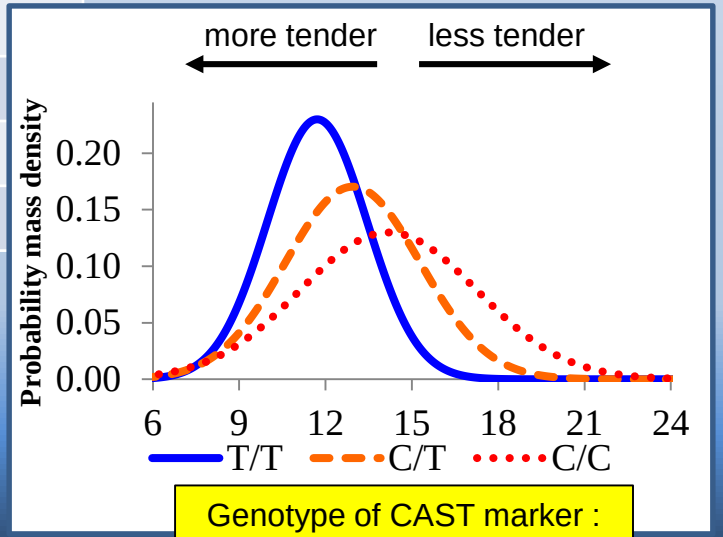
Creating a research population to assess genetics across the beef cattle industry

- **The Germplasm Evaluation (GPE) population at USMARC**



  - 18 breeds
  - Sampled to represent U.S. industry
  - Purebreds and crossbreds
  - Multi-generation pedigrees
  - Intensive and expensive traits

Markers for Beef
CAPN1 (tender)
CAST (tender)
GDF8-F94L (lean)
TG (fat)
DGAT1 (fat)
CSN1S1 (fat)
GHR (fat)



Genotype of CAST marker : TT more tender

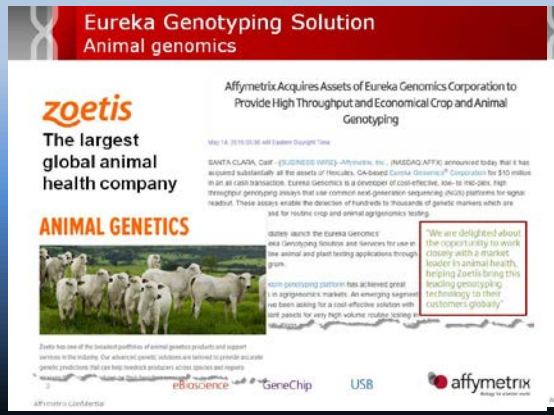
Using genomics and genetics to improve beef cattle

Progress :

- **Technology for re-sequencing has been acquired and optimized**
- **A reference panel of animals from top breeds has been established and sequenced**
- **Critical improvements to the reference genome assembly annotation and contiguity were initiated**
- **Methods to impute sequence in the GPE pedigree based on genotyping arrays have been developed**
- **Approaches to use sequencing of pooled DNA from case/control populations have been developed to support identification of variation in populations outside USMARC**
- **>120,000 industry animals have been genotyped**

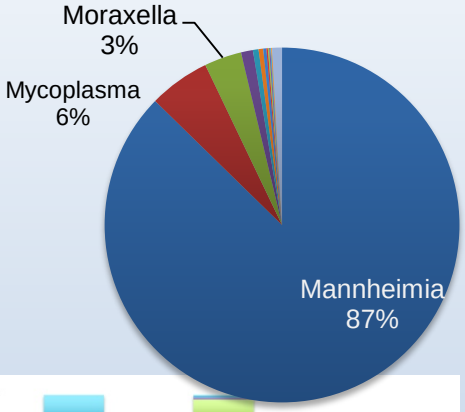
Products

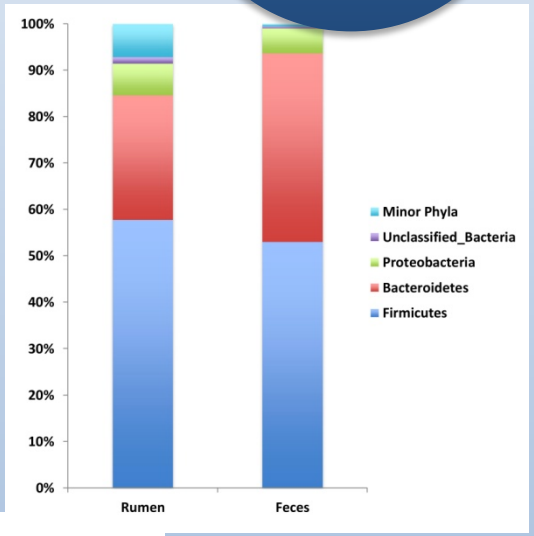
- **Across-breed EPDs**
  - Popular product extensively used by commercial producers to compare genetic merit of bulls across breeds
- **Breed differences and genomic marker effects for novel traits**
  - Reproductive success
  - Disease susceptibility
- **Graduate student training with partner universities**
  - 6 students since 2012
- **Low-cost genotyping system developed with industry partner**
  - CRADA with Eureka genomics

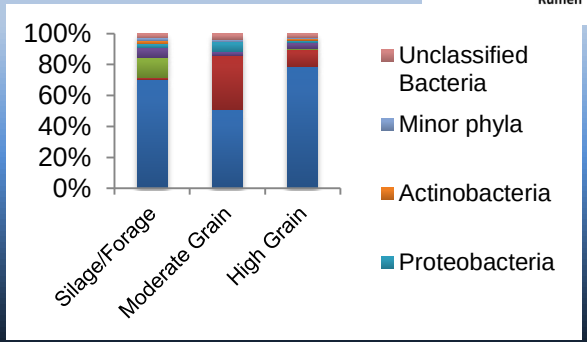


Studying the impact of microbial ecology on production efficiency, food safety, and animal disease

- Recent studies have revealed roles of variation in the “microbiome” in obesity, disease susceptibility, and pathogen shedding for humans and biomedical model organisms
- Cattle microbiome projects:
  - Compare rumen vs. fecal diversity
  - Characterize the gastrointestinal tract microbes in animals with high and low feed efficiency or methane production.
  - Characterize microbial populations of animals that shed pathogenic strains of *E. coli*
  - Compare microbial populations of the nasopharyngeal area in animals during disease progression to identify most likely pathogens, and potential antibiotic resistance
- Identified bacterial groups shifts due to diet and related to *E. coli* shedding
- Quantified diversity of rumen, lower GI, feces with different diets
- Developed database of sequence to use in comparing animals in respiratory disease studies







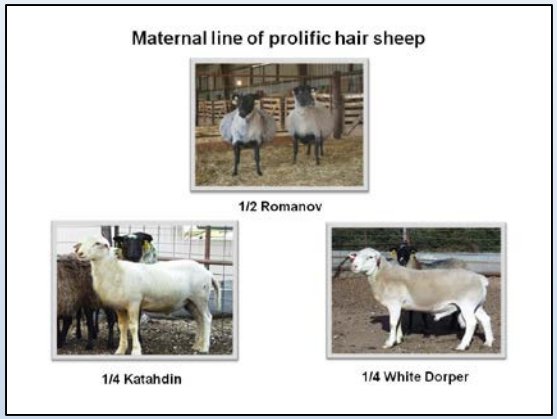
Genetic and genomic research in sheep

- **Industry issue :**

  intensive management of existing U.S. sheep breeds requires expensive labor, lowers profitability

  – USMARC solution : Develop and release new breed that doesn't require shearing or other intensive management

    - 22% more lambs versus industry standard breeds
    - Weaning rate 35-45% higher
      - Survival rate is 10-16% higher

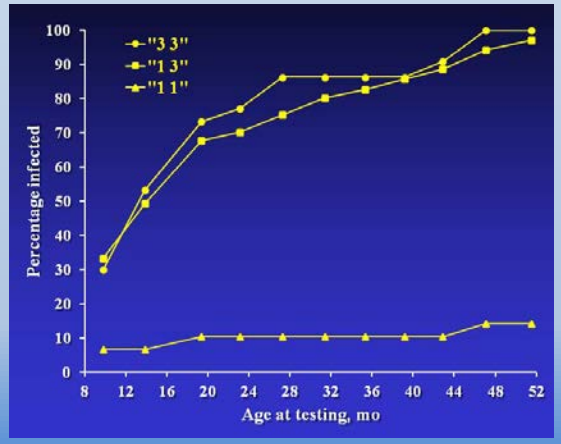


- **Industry issue :**

  Reduced productivity due to Ovine Progressive Pneumonia (OPP)

  – USMARC solution : develop and release genetic marker associated with OPP susceptibility

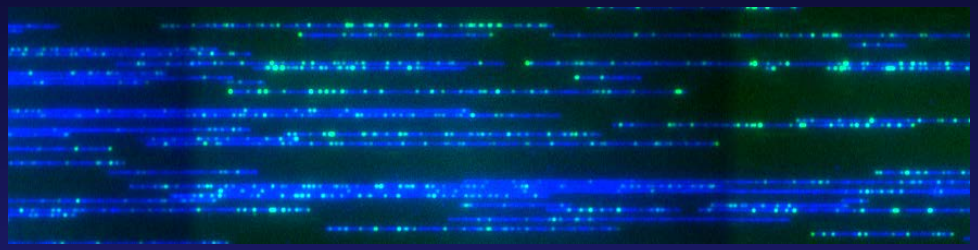
    - Dramatic reduction in OPP in selected animals
    - New models for OPP transmission developed

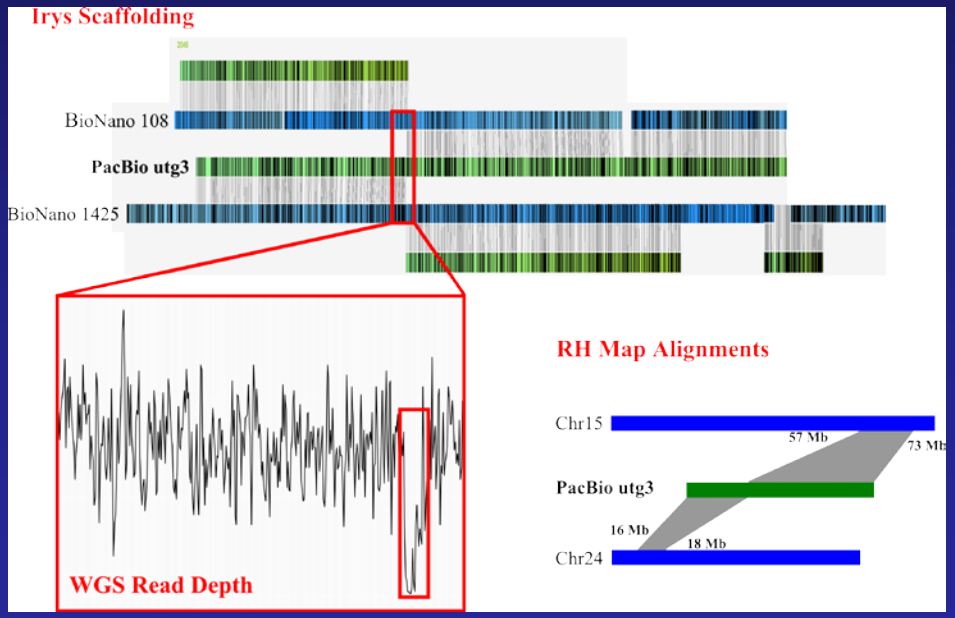


Assembling a new Goat Reference Genome

- Capra hircus
  - 2.8 Gbp genome
  - 30 chromosomes
- Project initiated under USAID Feed the Future
- Selected a highly inbred San Clemente Goat

Implementing Cutting Edge Technology to Develop Genomics Resources

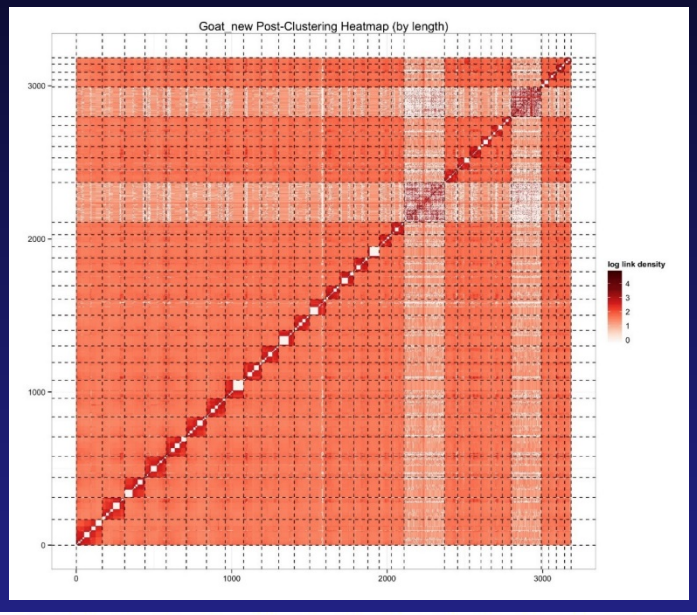


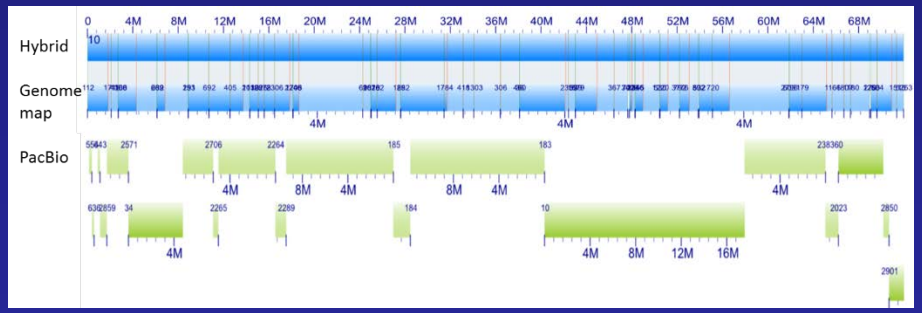


- Collaboration with NHGRI, University and Private researchers
- First use of PacBio-only reads + Optical Mapping + Hi-C data to develop a reference genome
- Solved new problems arising from the data

A New Paradigm of Genome Assembly

- Completed and polished in a fraction of the time previously needed
- 100 fold improvement of genome continuity over previous agricultural reference genomes
- Developed a protocol for the creation of new Reference genomes
- First single chromosome scaffold to be reported in the literature!

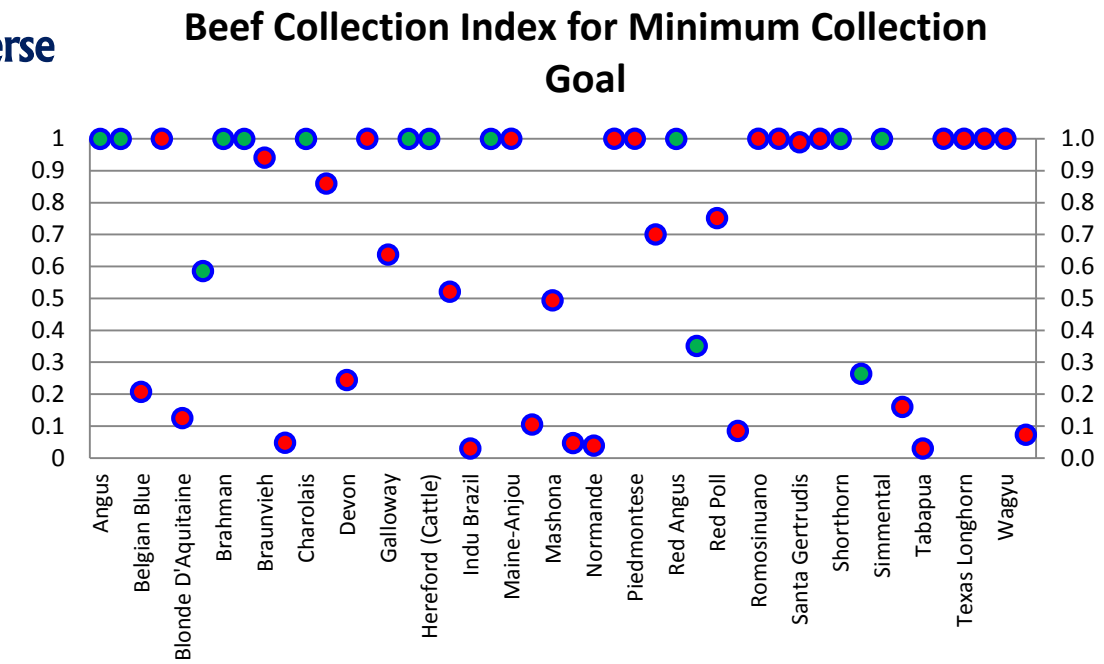




Safeguarding and Utilizing Animal Genetic Resources – Gene Bank Development and Use

World's largest and most genetically diverse collection of livestock genetic resources

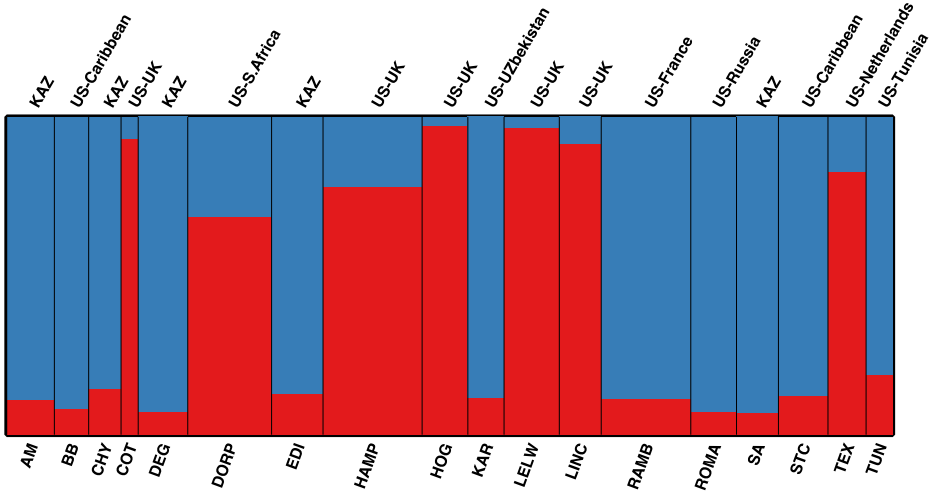
- 35 species
- 156 breeds
- 189 research and industry lines
- 29,167 animals
- 822,562 samples

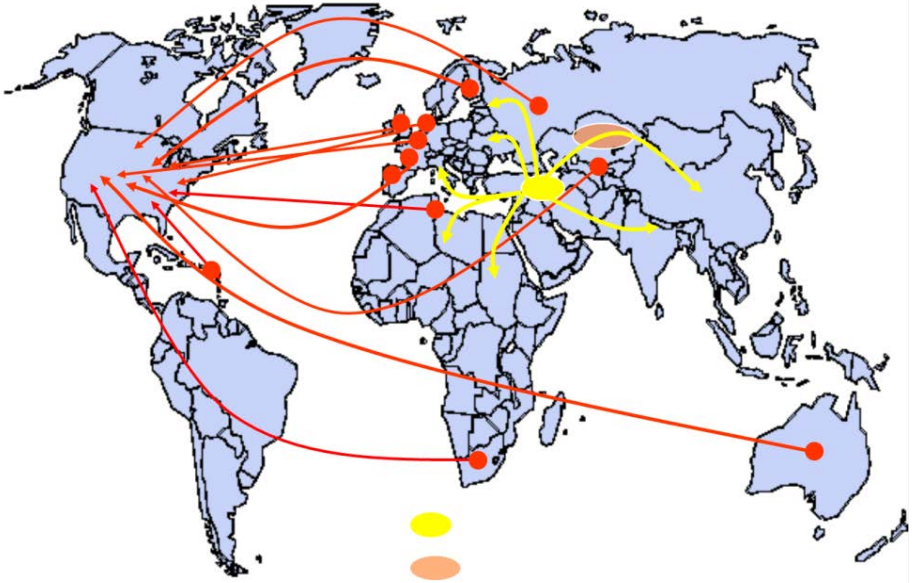


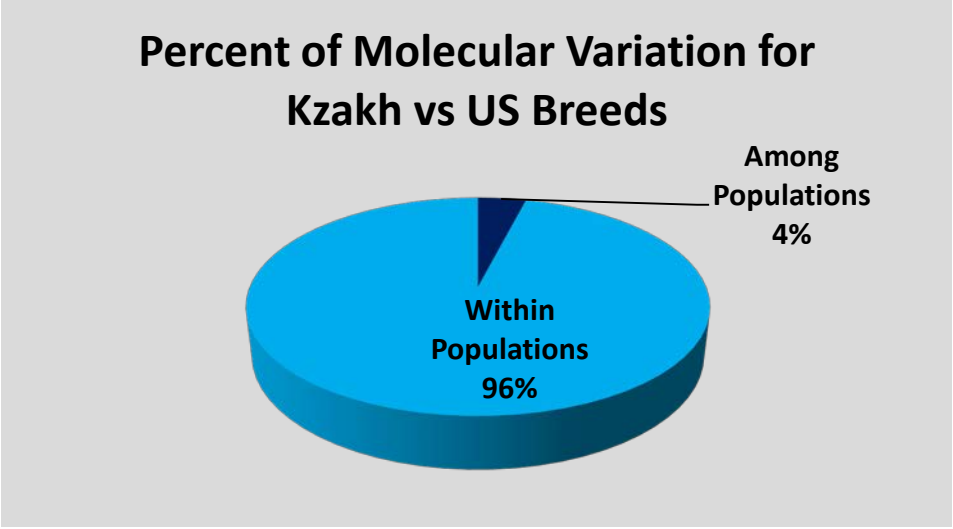
19,323 samples from 7,640 animals released for: DNA research, reconstituting populations or introducing genetic variability

Milking Shorthorn bull sired by repository bull. Breeder then submitted semen from progeny to NAGP

Safeguarding and Utilizing Animal Genetic Resources – Quantifying Genetic Variability



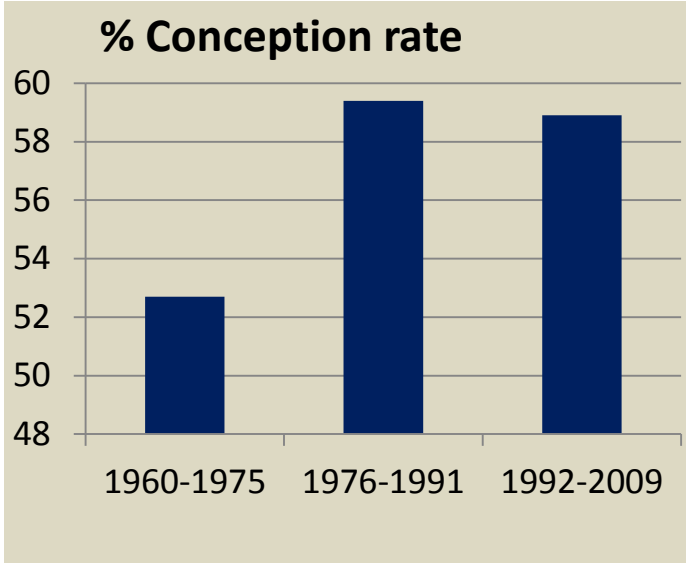




- Not all species are as highly structured as cattle.
- US has sampled widely making it a new center of genetic diversity
- As trade in genetic resources becomes more contentious our existing diversity should serve industry well

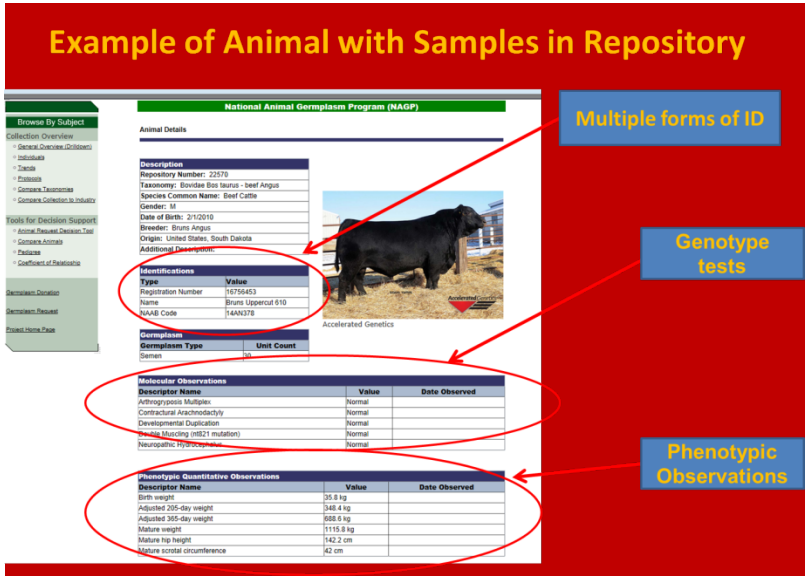
Safeguarding and Utilizing Animal Genetic Resources – Evaluation of Techniques for Conserving Tissues/Germplasm

New technique for harvesting, cryopreserving & transplanting ovarian tissue – initiates industry collection (saving $1million/yr/line) & speeds conservation of fancier breeds



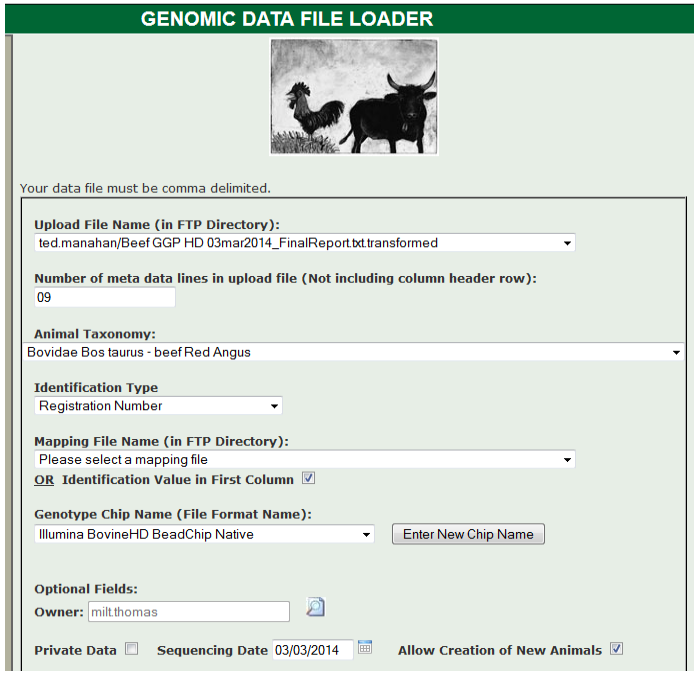
Evaluation of storage semen viability with LSU – no significant effect of storage time

Safeguarding and Utilizing Animal Genetic Resources – Animal-GRIN Database + Genomics Database



Animal-GRIN expanded to include storage of genomic information, why?

- Increases the value of samples in collection
- Lack of industry curation
- Research community lacks a mechanism for long term storage of data after grant is completed



Animal-GRIN is a web based, publicly accessible database used by NAGP, Brazil, and Canada

Information in Animal-GRIN: Sample information, storage, phenotypes, environment, management & genetic tests (e.g., halothane in pigs)

Version 2 of Animal-GRIN developed and in production

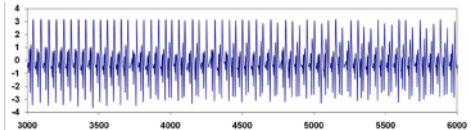
Hits: 2013 – 574K; 2014 – 814K

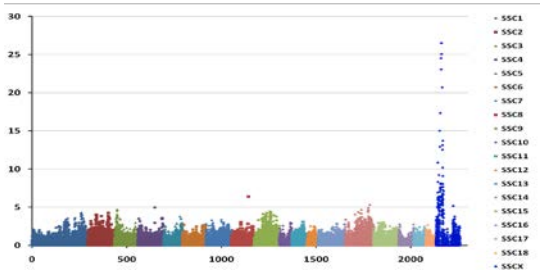
Transportation Losses in Pigs

Problem: **Transport losses cost the pig industry over $50 million/yr**.

Accomplishment

- A novel stress syndrome arose in the USMARC commercial population during transport.
- A special population of animals was created and detailed physiological traits measured.





- Genetic mapping determined that the defect was caused by a mutation in the dystrophin gene.
- Dystrophin mutations cause muscular dystrophy in humans.

- These pigs have been transferred to research labs that are working to develop therapies to mitigate the devastating effect of muscular dystrophy in children.

Genetics of Pork Quality

Problem: **A dissatisfied eating experience will decrease demand**.

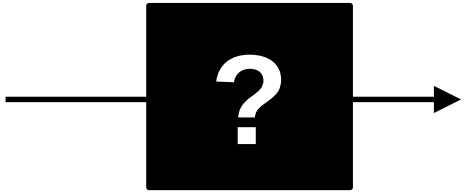
Accomplishments

- The results of genome scans within the USMARC population have been leveraged with data from other research populations to identify more robust QTL.
- These results are then confirmed in commercial populations by testing pork samples directly from commercial processors.

- Genetic markers developed for calpastatin, which affects tenderness, are commercially available.
- A meta-analysis of 3 unique populations yielded associations with pH and water-holding capacity.
- Studies on color stability have also produced interesting results that may allow a longer shelf-life of the product.

Genetics of Sow Lifetime Productivity

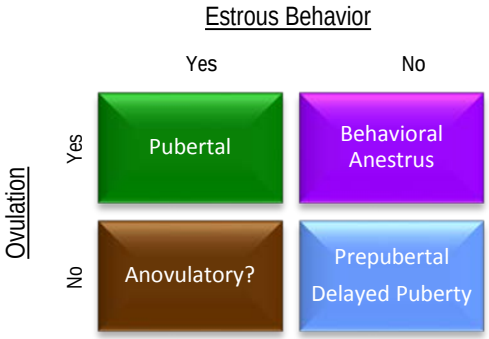
Problem: **How do you select the most productive sow at an early age?**



Genomics!

Puberty Development

- Individually study ovulation rate and expression of estrus.
- Identified candidate genes associated with puberty and failure to reach puberty.



Sow Retention

- Identified genes important for return to estrus in first parity sows.
- Combining data from over 1,000 USMARC sows and 3,000 commercial sows to understand retention patterns of sows through 4 parities.

Prolificacy

- Conducted genomewide scans for regions associated with litter size born, farrowing speed, stillborn number, ovulation rate and birth weight.
- These results will be validated in data collected at commercial farms to evaluate their utility to the swine industry.

Goal - Sustainable Poultry Production

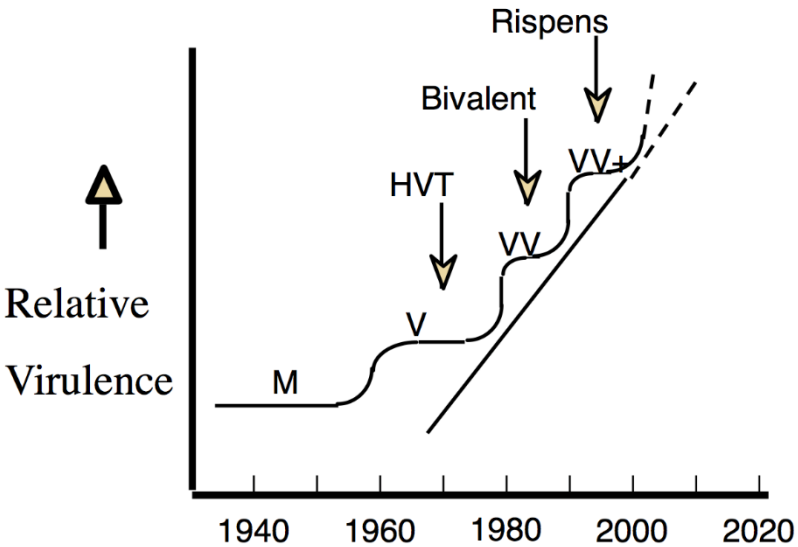
Growing Importance of Animal Health

Chick-fil-A

our journey: ANTIBIOTIC-FREE CHICKEN

Goal - Sustainable Poultry Production

Marek's Disease - #1 Chronic Infectious Disease Concern for Poultry Industry



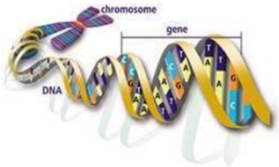
- A T-cell cancer caused by the highly oncogenic Marek's disease virus (MDV), an α-herpesvirus, which is ubiquitous in the environment.
- Marek's disease costs the worldwide poultry industry $1-2 billion per year.

- Vaccines inhibit tumor formation but do not stop viral replication and spread, and are the likely force for viral evolution
- **Need a sustainable alternative solution to widespread vaccination**

Why Improve Genetic Resistance?

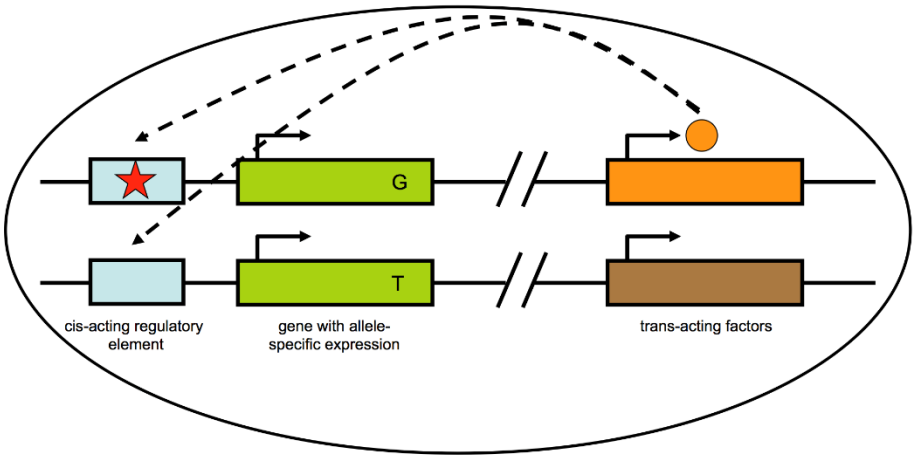
- It works!
- Genetic change is permanent, thus, requires no further input once established
- Augments vaccinal control measures
- Fits very nicely into the new paradigm of using genomic selection
  - No need to expose elite flock to hazardous pathogen
  - Select both sexes at hatch
  - Higher accuracy and can be combine with other traits

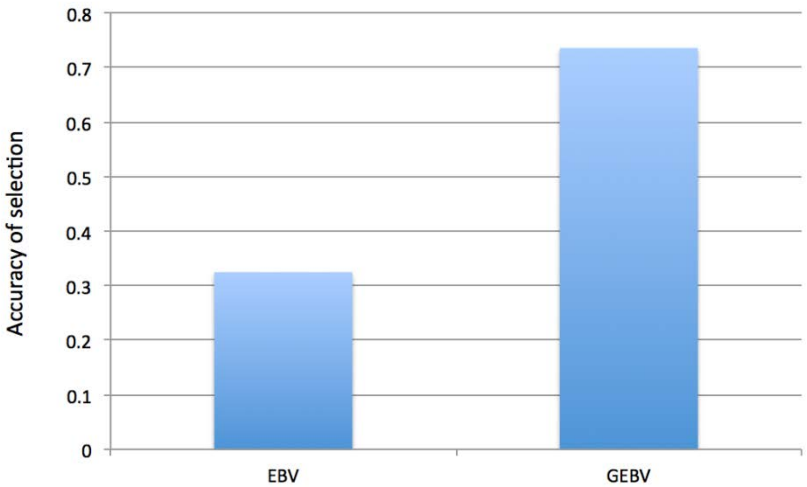
Goal - Sustainable Poultry Production



Genomic (DNA-based) selection for enhanced genetic resistance to Marek's disease

Allele-Specific Expression





- Genetics markers were screened to identify differences in gene expression in response to viral infection.
- These SNPs account for 83% of the genetic variance.

- Genomic selection is 125% more accurate compared to state-of-the-art breeding methods.
- Other advantages of genomic selection are birds can be tested at day of hatch, both sexes can be screened, no need to measure traits.

Develop and Implement Genome-Enabled Genetic Improvement Programs

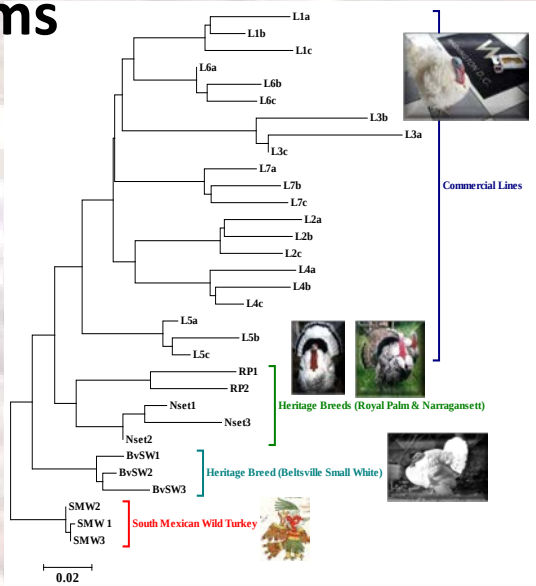
Problem: Genetic improvement for turkeys has been achieved using selective breeding programs based on physically-measureable traits. For less measurable traits such as immunity and reproduction, more sophisticated genomic selection is needed. While genomic resources are available for chicken production, these tools have not been available to the turkey industry.

Accomplishments: Multi-platform next generation sequencing of the domestic turkey genome and development of a new genetic tool (SNP array) for turkey producers.

ARS scientists, in partnership with VPI and UMD, sequenced and annotated the genome of the domestic turkey using a unique sequencing strategy of data from both Roche 454 and Illumina GAII platforms.

ARS scientists conducted a broad assessment of 11 different turkey lines and breeds and discovered 5.49 million SNPs that are potential markers for genetic improvement.

Impact: A comparative analysis of the turkey, chicken and zebra finch genomes supports the characteristic stability of avian genomes and identified genes unique to each species and the avian lineage. An array containing *60,000 of the best genetic markers was made publically available* for turkey producers and researchers.



Phylogenetic tree of 7 commercial turkey lines and 4 turkey breeds, including the ancestral South Mexican wild turkey.

Domestication of the commercial turkey: a true U.S. immigrant.

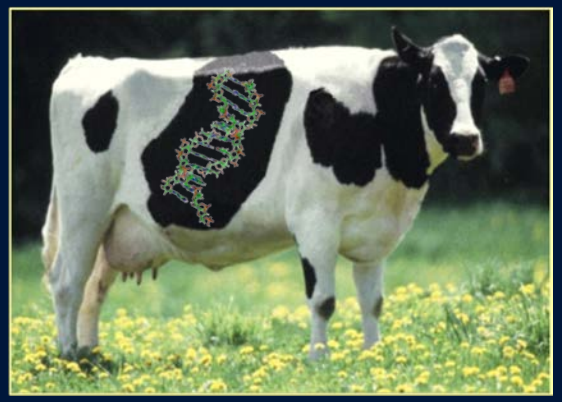
Genomic predictions for global dairy cattle developed by USDA and transferred to the industry

From research development …

… to service implementation

A project to improve health and productive efficiency of dairy animals through genetic evaluation, thus improving the global diet

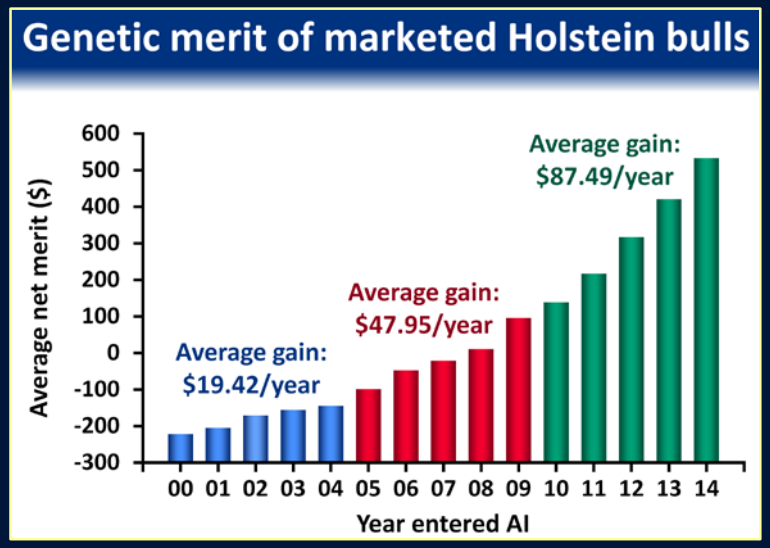
Accomplishments



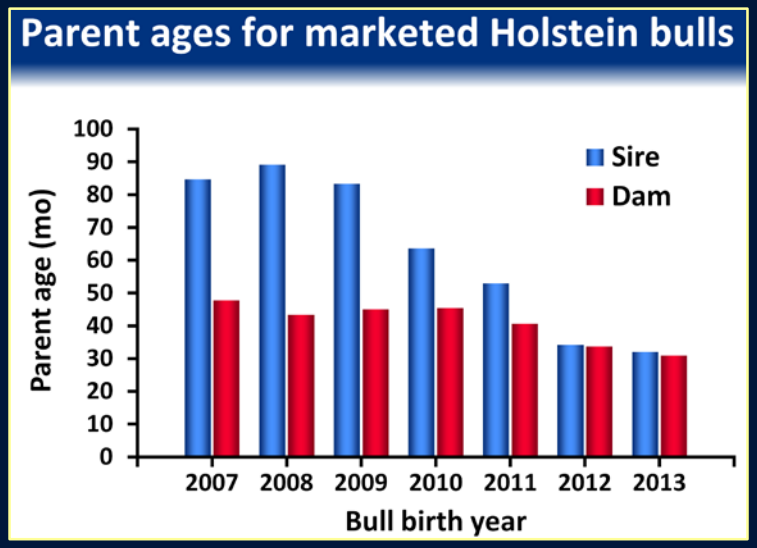
- Development and implementation of a genomic evaluation system
- Creation and enhancement of a genotype database that now includes over 1.2 million animals from 50 countries
- Collaborative development of genotyping tools
  - Growth from 1 chip to imputing 19 chips of many densities
  - Addition of sequence data
- Development of methodology that is used globally to compute genomic predictions
- Transition of (1) data collection and storage and (2) calculation and distribution of evaluations to industry

Impact

- Faster genetic progress



- Shorter generation interval



- Healthier, more productive dairy cattle with a world-leading genomic prediction system and vast database that producers in many countries now use routinely to improve their herds for many traits

Questions on Component 2?

Component 3: Measuring and Enhancing Product Quality and Enhancing the Healthfulness of Meat Animal Products

- Problem Statement 3A: Systems to Improve Product Quality and Reduce Variation in Meat Animal Products.

- Problem Statement 3B: Improving the Healthfulness and Nutritional Value of Meat Products from Traditional and Non-Traditional Production Systems.

LED beef carcass grading camera system

Problem

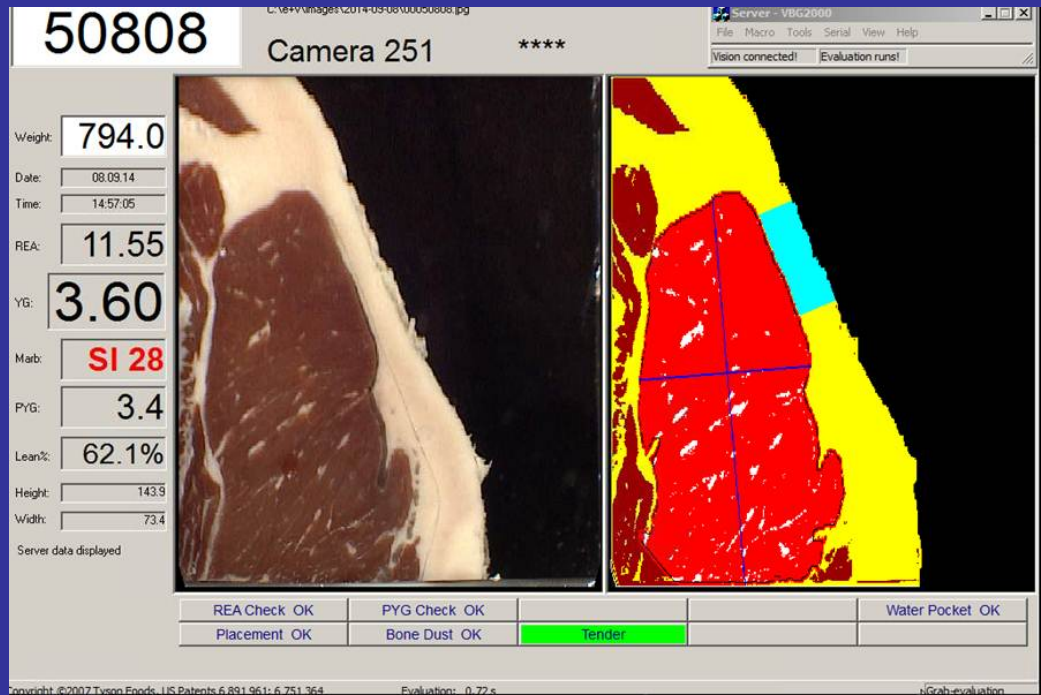
The USDA-AMS-approved beef grading camera included a xenon flash tube which was sensitive to temperature and fatigue. The instrument manufacturer designed a new camera system with LED lamps which provide a more consistent light source. However, because the LED lamps changed the color pattern of the resulting images, the LED cameras could not be implemented without validation of the accuracy of regression equations under these new conditions. Industry also demanded that we evaluate the ability of this system to predict tenderness.

Results

Equations for grade traits and slice shear force (tenderness) were developed, validated, and approved by USDA-AMS.

Impact

Approximately half of the fed-beef packing plants in North America have implemented (or upgraded to) the LED camera. Tenderness prediction is used in two plants so far.



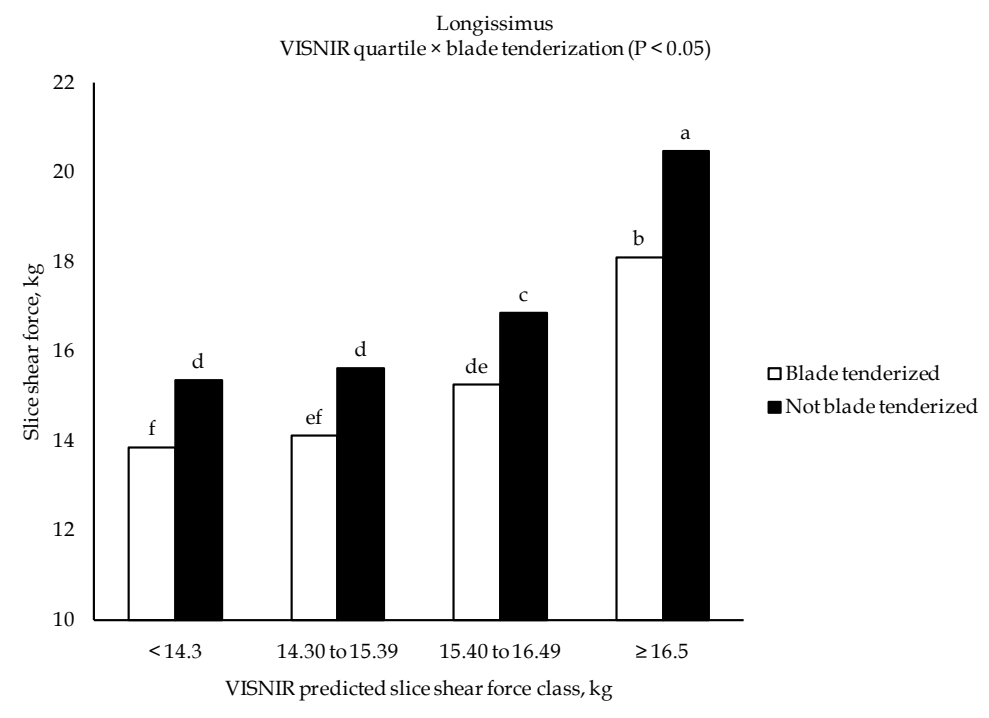
Interaction of tenderness prediction classification, aging time, and blade tenderization on tenderness of strip loin and top sirloin steaks

Problem

Many food service entities have contemplated eliminating the use of blade tenderization, due to pathogen risk. Thus, alternate strategies to ensure tenderness are needed.

Results

Determined that optimal aging times vary depending on tenderness classification and blade tenderization for strip loin and top sirloin.



Impact

USMARC tenderness prediction system could be used to ensure tenderness without blade tenderization.

Chilling rate effects on pork loin tenderness in commercial processing plants

Problem

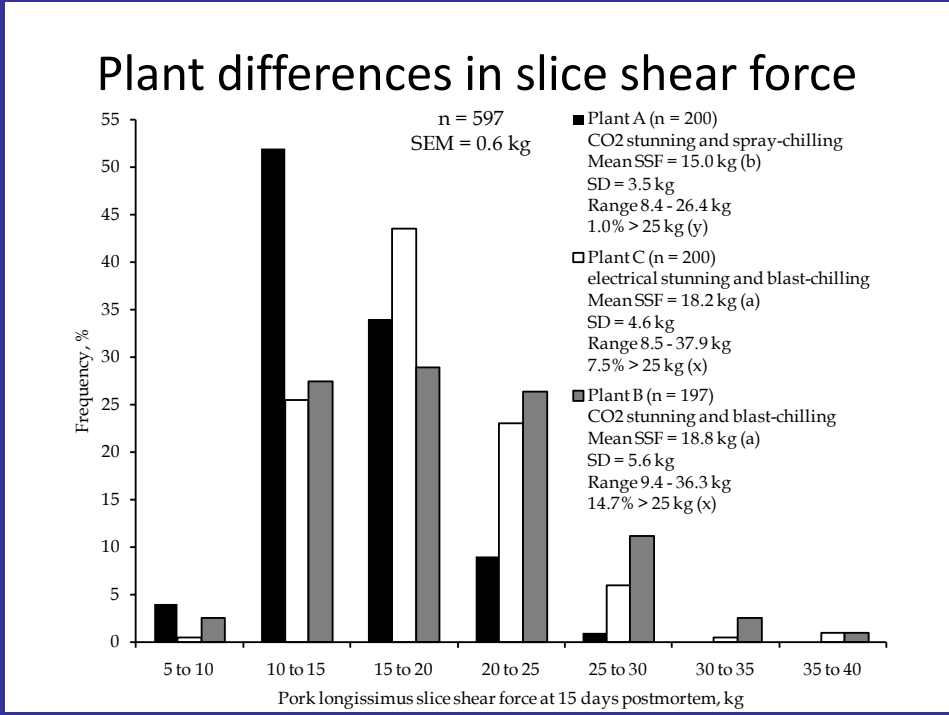
Major tenderness differences observed among pork packing plants. Objective evaluation was needed to determine the source of plant differences.

Results

Determined that extremely fast chilling systems result in less tender pork loin chops.

Impact

National Pork Board convened its Pork Quality Solutions Team to make the industry aware of this issue. This led some companies to test strategies to optimize chilling and tenderness has improved.



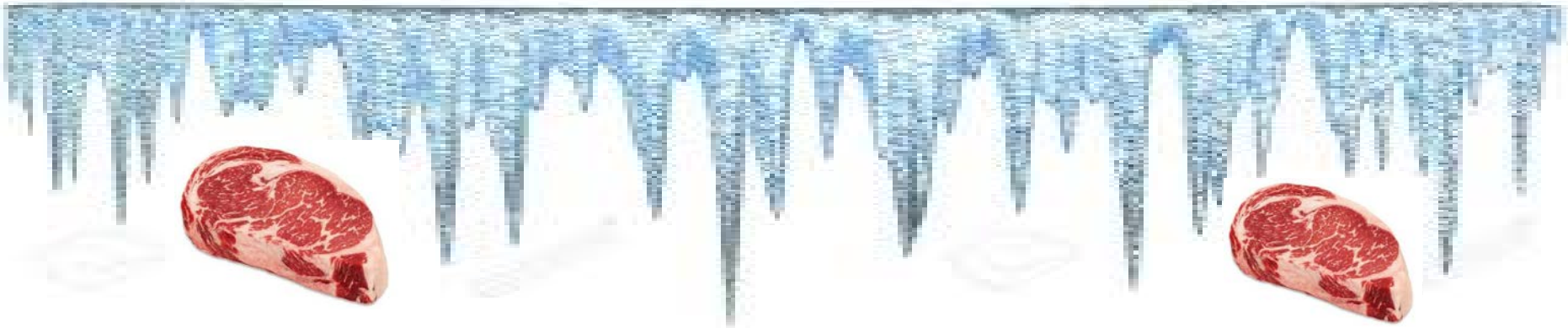
Understanding/Improving Lean Color Stability

Problem

- Premature browning of retail meat products costs the meat industry more than $1 billion annually when product has to be discounted.
- Previous research had minimized animal effects, but meat cuts from some carcasses fail to meet specifications for case-ready programs.

Results and Impact

- Demonstrated animal-to-animal variation in lean color stability.
  - Contrary to previous wisdom. Researchers have changed approach as a result
- Characterized genetic effects of sire breeds on lean color stability of ribeye steaks.
  - First report of genetic effects on lean color stability in a population reflecting the U.S. beef herd.
  - Indicates opportunity for genetic selection
- Developed non-invasive technology to predict lean color stability.
  - Provided the industry a tool to manage the problem
- Identified previously unknown mechanisms contributing to lean color stability variation.
  - Greater understanding of the biology of the trait
  - Further influenced research in the field



Improving Meat Tenderness with Freezing

Too much variability in eating quality, especially tenderness, due to numerous breeds, production systems, growth technologies, and postmortem processing variables.

Determined freezing can improve tenderness 2 ways:
Direct impact of structural damage from ice crystals,
Reduces the protein that inhibits tenderization during aging.

We have been working with a beef processing company that is interested in implementing the process, to optimize the freezing/thawing/aging parameters.
Will result in consistently tender meat.

Questions on Component 3?

Panel Discussion