W3173: Impacts of Stress Factors on Performance, Health, and Wellbeing of Farm animals (from W2173)

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Statement of Issue(s) and Justification:

By 2050, the world population will be 34% higher than today, reaching an estimated 9.1 billion people. Food animal production will need to increase by 1.7-fold to meet the growing world demand for animal protein (FAO, 2012). As a result, animal husbandry systems may become more intensified (concentrated animal feeding operations; CAFO) because fewer natural resources will be available to raise animals. In addition, new biotechnologies (e.g. gene-editing, growth promotants, feed-additives) and better housing systems may be needed to help increase the adaptation rates for domestic animals so that they can remain efficient and healthy, despite a variable climate. However, CAFOs and biotechnologies are often viewed by consumers with animosity over the concern of animal welfare and food safety, therefore, producers have to balance these concerns with productivity despite an increase in climate variability and limited resources. Nonetheless, animals in both intensive and extensive production systems are faced with potential macro- and micro-environmental stressors (Canario et al., 2013). In order to balance these challenges, producers will need to raise and manage farm animals so that they are resilient to environmental stressors. Stress physiology plays a significant role in objectively improving animal welfare, productivity, and ultimately, food security. When animals are not able to cope with macro- and micro-environmental stressors, US livestock producers can lose billions of dollars. For example, St. Pierre et al., et al. (2003) reported that without heat stress abatement measures, total losses in poultry and livestock costs $2.4 billion each year. Dairy cattle were the most affected in this calculation, with a yearly loss of $897 to $1500 million annually (St.-Pierre et al., 2003). In 2003, Mader predicted that the extreme climate variation will cost beef cattle industry members $10-20 million annually.

Climatic conditions affect both animals in both CAFOs and extensive systems (Renaudeau, et al., 2012) In 2013, extreme cold fronts in October eliminated or killed over 10,000 beef cattle in extensive systems across the north and Midwest US regions. In winter 2015, 30,000 dairy cows reared in intensive systems in TX and NM were killed after an unusual winter storm, “Goliath,” hit the region. Severe droughts caused overall cattle numbers in 2013 to reach their lowest since 1952 (Stewart, J. 2013). The extreme weather changes reduced the amount of beef cattle produced in recent years. The loss in cattle numbers tripled the male dairy calf market, and are typically produced and managed in CAFOs (Hulbert and Moisá, 2016). Poultry and pig CAFOs were also affected by climate change, as buildings are difficult to keep efficiently cooled and ventilated during heat waves. In addition, droughts changed in wild-bird migration patterns, and incidence of

disease in poultry and swine may increase (Arrus et al., 2006; Tian et al., 2015). Animals will need to be selected for stress-resilience, immunocompetence, and performance, even though these three traits do not always complement each other. Management stressors can exacerbate diseases caused by macro- and micro-environmental stages, especially during early stages of life (Turner et al., 2001).

The research, technologies, and information generated by the previous W1173 and W2173 groups provided both basic and applied knowledge to our stake holders. This group has succeeded in sharing resources and creating novel methods to improve stress-resilience and immunocompetence in animals. For example, this group is responsible for many of the heat stress abatement technologies and basic understanding in improving cattle comfort and health. They (1) produced new technologies to measure and better understand animal stress responses, immunity and behavior; (2) identified genes and gene expression for stress-resilience and immunocompetence and, (3) identified key management and nutritional strategies to help animals acclimate to environmental changes. These collaborative efforts will need to be accelerated and continue to improve food security from the animal sectors. Multistate efforts allow for the sharing of data, resources, animals, and ideas. These scientists and engineers pull together a diverse range of skillsets and expertise to address the objectives outlined in this proposal. Thus, the outcomes of this multistate project will broadly impact production practices, animal comfort and wellbeing, and improve profitability across diverse livestock commodity sectors.

Accomplishments for Objective 1: Identify measures of animal stress and well-being and characterize factors affecting the biology of stress and immune responses

Multispecies applications

Collaborations (MS, TX, TN, VI, HI, AZ, NY) collaborations conducted cooperative biophotonic studies in livestock (bovine, equine, ovine, avian) to provide meaningful and objective measures of production and stress physiology. Investigations included using thermography technologies to detect in lameness detection in ungulates and energetics in birds. In addition, thermal signatures relative to identify genetics and hair coat in beef cattle were evaluated. In vivo, real-time imaging of experimentally induced mastitis, reproductive tract infection, and antibiotic efficacy was modelled and assessed using thermography and laparoscopic technologies were studied. The portable evaporative machine developed from the previous W2173 projects (Vapo meter, Delfin Technology, Kuopio, Finland) and the bovine portable evaporative machine (Gebremedhin et al., 2008) were adapted for use in sheep that were pregnant or open and provided shade or not provided shade. In addition, Missouri used numerous physiological measures of thermal status to compare responses of cattle to chamber stress tests and naturally occurring field conditions. Telemetric temperature transmitters in the rumen of each animal monitored core temperature. Some variables (e.g., feed intake, sweat rate) showed no change between tests, while others (e.g., rectal temperature) showed adaptation. Ultimately, a new thermal strain index for multiple stressors will be developed that identifies real world adaptation.

Swine

Pre-weaning mortality remains both an economic and a welfare issue on swine farms. A large variation in piglet mortality exists among sows, with some sows (high risk sows) losing more piglets than others. To characterize these high risk sows, hormonal differences between high risk and low risk sows were compared. Minnesota evaluated the effects of social stress of sows during gestation on behavior and well-being on offspring heart rate, peripheral cortisol concentrations, fear response, and measures of performance. Results indicated no association of high risk and low risk sows with serum prolactin, cortisol, or urocortin concentrations. However, high risk sows had higher oxytocin concentrations compared to low risk sows. **T**his study begins to identify stress-measure differences between sows that have a higher pre-weaning mortality to those that have a lower pre-weaning mortality in a group-farrowing system. The results help us understand the maternal biology that contributes to piglet survival. In addition, MN evaluated social rank by measuring agonistic behavior outcomes of 120 gestating sows during initial commingling. Low ranking sows tended to have smaller litter size at birth than high ranking sows. Similar studies were performed at a large-scale commercial swine unit. Sows in group-housing systems had higher culling rate due to poor body condition and injuries resulted from aggression than sows in individual stalls.

Recently, there has been a push to change sow housing from gestation stalls to group housing. Several different types of systems have been investigated including free access stalls and electronic sow feeding stations. With any management system, day-to-day situations need to be studied and resolved in the best possible manner. Several studies have been conducted to solve such issues. One study determined all sows access to feeding stalls through out the day provided hiding spaces by low ranking sows thus reducing fighting and improving animal well-being. The next study focused on management of electronic feeding stations (EFS). It was determined that sows in a static group sustained fewer skin lesions and had less incidence of lameness before farrowing than sows in dynamic group. However, sows that were moved to the EFS at the pre-implant stage lower farrowing rate (82.3% vs. 86.7%), but had fewer skin lesions before farrowing compared sow moved post-implant. Neither social management nor stage of gestation at mixing affected total born, born alive or still-born litter sizes. In comparing the ESF to stalls, in general, sows group-housed with ESF performed similar to but sustained fewer skin lesions than sows in stalls.

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Collaborators (IN, KY) examined the effects of feeding a diet supplemented with a protected fish oil (PFO) supplemented product enriched in omega-3 polyunsaturated fatty acids to gilts/sows beginning approximately 2 wk prior to farrowing and up to d 16 ± 2 of lactation on modifying the fatty acid composition of the mammary secretions. Results from this study showed a significant increase in the level of EPA and DHA fatty acids measured in colostrum and milk samples collected from sows fed the 1.0% PFO supplemented diet versus the diets supplemented with 0, 0.25 or 0.5% PFO. A subsequent study is ongoing to determine if dietary supplementation of PFO reduces the pig's physiological stress response due to weaning.

Semen ejaculates contain variable populations of spermatozoa that significantly influence the fertility outcomes of semen doses used for artificial inseminations (AI). The routine evaluation parameters of semen quality are not fully reliable to predict sire fertility. Researchers (MS) took advantage of the recent progress in nanotechnology by combining this technology to reproduction. Nanotechnology refers to the study of particles of less than 100 nm in size, which allowed us to design specific iron oxide-based nanoparticles as new tools for high-throughput improvement of semen quality. This approach uses magnetic properties to attract spermatozoa bound to specifically designed-magnetic nanoparticles while the non-bound "viable" sperm are eluted and used for inseminations. Boar semen was obtained in artificial insemination doses from a local boar stud. Doses were mixed with or without magnetic nanoparticles designed to target and deplete moribund and poor performing spermatozoa under an electromagnetic field. Eluted semen had greater sperm motility characteristics and resulted in normal pregnancy rates. Interestingly, piglets produced in the nanopurified group comprised of 55% males compared to 38% in the control group; which requires further study whether gender is skewed in this process. Magnetic nanoparticles used in this preliminary study exhibited no toxic effects on sperm fertilization capacity and piglet viability.

Cattle

Collaborators (NY, HI, MS) evaluated practical heat stress abatement (THI > 86.5) methods (wetting hide and fans) respiration rates (RR), single-time point rectal temperature, automated vaginal temperature, and dorsal and udder skin surface temperature using a novel technology of digital infra-red thermometer and infra-red thermography. This study had several outcomes: Wetting the hide or udder reduced RR, which reduced further with the addition of fans; However, the addition of the fans only reduced core body temperatures in hide-wetted cows. There was also a close correlation between virginal and rectal temperature (R^2=0.93), therefore, vaginal temperatures may replace the rectal temperature monitoring. Collaborators (USDA-IN, FL) studied cow's comfort on either rubber or concrete flooring. They measured lactation performance, locomotion, hoof health, measures of immunity and stress. Concrete-housed cows had measures of immunity indicating chronic pain, which increased throughout lactation and exposure to concrete flooring.

Environmental determinants of core body temperature (Tcore) and feed intake (FI) were determined in crossbred Angus (Bos taurus) steers. Novel technologies were shared and developed

in this multistate project. For example, Tcore using intrarumenal telemetric boluses (SmartStock, Pawnee, OK) and electronic ID tags (Allflex US Inc., Dallas-Fort Worth, TX) connected to a GrowSafe FI system (GrowSafe Systems Ltd., Airdrie, AB, Canada) provided FI. In addition, ambient temperature and humidity were measured (Hobo H8 Pro; Onset Computer, Bourne MA) and black globe temperature (BG) was measured. Temperature-Humidity Index (THI) was calculated for all combinations. Best FI predictor, based on highest R2 was mean THI using BG in the sun, with a 3-day delay (R^2=0.24). Analysis of Tcore used group averages for each hour. Both regression analyses identified Ta alone with no delay as the best indicator of Tcore (R2=0.62). These results indicated that although air temperature alone may be the best predictor of core temperature in the heat, radiant heat load may be a better determinant of feed intake over time. In field studies of Angus cattle, air temperature alone may be the best predictor of core temperature in the heat, and radiant heat load may be a better determinant of feed intake over time.

The hair coat of animals plays a critical role in heat and moisture transfer from the skin surface. Endotherms interact with the thermal environment that affects their growth, production and reproduction potential at the skin-hair coat interface. Animal hair-coat traps air to provide insulation from cold but becomes an obstruction for evaporative cooling by reducing the velocity and moisture gradients through the fur layer in hot and humid conditions. Hair coat properties are inputs to models of bioenergetics (heat and mass balances) of animals. It is therefore imperative that physical and optical properties of the hair coat are accurately characterized for modeling heat and mass transfer through the hair coat. In addition, hair coat properties can provide information of the breed of animals that are more likely to adapt to a particular environment. Availability of physical and optical data in literature is sparse at best; what is available is mostly from studies done in the early 1950's to 1970's which do not represent present-day high milk volume-producing cows and changes in nutrition of animal feed. Biological rhythms of core body temperature of beef cattle were evaluated with respects to breed and hair coat color (NE; UN and USDA-MARC). Despite shade-availability, dark-coated cattle had higher day time temperature. Nonetheless, dark-coated cattle were capable of returning to normal temperatures by early hours of the day. Low thermal neutral temperatures were not different between breeds. The cattle with dark hair coat are most susceptible to heat stress conditions and there is a potential need to provide shade to alleviate the stress. To compliment this work, the NY-group, supported by a USDA-NIFA-TSTAR program, developed the characterization of the physical and spectral properties of animal hair coat and their effects on energy balance of cattle exposed to solar load. Livestock in the tropics tended to have shorter hair coats than their temperate counterparts. Comparisons of heat tolerance of Charolais-sired, slick and normal-haired 25% Senepol cattle of the same breed composition showed that slick-haired animals were able to maintain rectal temperatures approximately 0.5º C lower than those of normal-haired animals under heat stress. Possessing a short hair coat may impact the behavior of cattle through their ability to tolerate high heat and humidity. The impact of the 'slick hair gene' on hair-coat characteristics and thermoregulation of Senepol cattle were evaluated. This research revealed that Senepol cows that are homozygous for the slick hair gene have lower respiration rates and lighter hair weights than non-homozygous cows. The physical

properties of the hair and hair coat were different with respect to body locations of a cow. The spectral properties (absorptivity and transmissivity) are profoundly different for the different hair coat color with absorption coefficient ranging from 0.98 for Black Angus to 0.92 for dark-red to 0.75 for tan, and 0.37 for white Charolais.

Collaborations (FL, OR) were established to improve upon a widely utilized biochemical assay for the measurement of haptoglobin in bovine plasma as a biomarker of stress and sickness. This collaboration developed and validated a quantitative mechanism for accurately measuring the mass of haptoglobin in bovine plasma, which many laboratories now utilize as part of their research in stress physiology and sickness. This work was very complimentary to the collaborations between USDA-TX and TN, as they isolated, purify and created an ELISA assay for bovine corticosteroid-binding globulin (bCBG). Furthermore, Gene expression of over 80 genes after handling and transportation of cattle was evaluated by Texas A&M. Fifteen genes were expressed at 3 and 4 days after transit. At just 1.5 h after handling, another set of 5 genes significantly increased in expression after 1.5 hours of handling alone (none transported animals). This complemented work with acute-phase proteins at other stations (Texas Tech, Oregon, etc.) and resources were shared to establish novel biomarkers to further distinguish acute from chronic stress in animals.

Senior bulls (TN, KY) were evaluated for temperament at the beginning and end of an 84-d test period. Data collected on exit velocity, chute score along with hair samples are currently undergoing analyses. In addition, Collaborators (MS) had the objective to evaluate the relationships among hair and serum cortisol concentrations and cattle temperament using previous methods and technologies established by our group. Spring born crossbred beef heifers were evaluated for temperament preweaning and at weaning by pen score (PS; 1 = calm and 5 =excitable), exit velocity (EV; m/sec) and temperament score [TS = (PS + EV)/2]. Serum cortisol moderately correlated with TS pre-weaning and at weaning and was a better predictor of temperament assessment than hair cortisol concentrations from either the shoulder, rib or hip areas at weaning. Thermal images of hair coat and skin assessments are currently being analyzed.

In Montana, most beef cattle are fed hay for 3-5 months each winter, which is typically a rancher's highest variable cost in their operation. Without feeding, beef cattle may lower their metabolic rates (MRs), similar to wildlife, without impacting reproductive performance. The objective was to determine if MRs of beef cattle track short-term (four days) step-wise decreases, then increases in feeding level. Over a 30 day period, MR were determined (based on O2 consumed) and respiratory quotients (CO2 respired/O2 consumed) of six non-pregnant, non-lactating Black Angus cows. Initially, cows were fed chopped hay (7.5% CP) ad libitum for 16 days to determine full feed (100%). Beginning on d 0, three cows were exposed to a Moderately Restricted (M) feeding level (110%>110%>55%>110%>110%), three cows were exposed to a Restricted (R) feeding level (110%>70%>35%>70%>110%) in four-day increments. Metabolic rates of all cows were measured the morning after each four-day, step-wise decrease and then increase in feeding level. Metabolic rates tracked feeding level. As expected, respiratory quotients declined as feeding

levels were restricted indicating that cattle were catabolizing fat. Overall, these results suggested that cattle entering winter in good body condition which are not fed hay during winter, but just graze, may conserve energy by lowering their metabolic rates.

Neonatal calves

The effects of pain control (meloxicam) on bull calves were evaluated after they were castrated at ages birth or at weaning (AR). Calves castrated at birth spent the least amount of time standing than calves castrated at weaning. Calves castrated at weaning and provided meloxicam spent more time standing than compared to weaned-calves castrated without giving meloxicam. Castration near birth did not impact weaning weight, behavior, or acute phase response; whereas, delaying castration until weaning reduced post-weaning average daily gain, but this reduction was mitigated by the use of meloxicam at the time of castration. Oral meloxicam reduced serum haptoglobin when administered to calves castrated at weaning, but not in calves castrated near birth. Therefore, oral administration of meloxicam may be efficacious when surgically castrating older bull calves at or beyond the typical weaning age.

Similar to the MN-pig studies, collaborators (USDA-IN, HI) evaluated how prenatal allostatic load (heat and other stress) affects bacterial levels and innate immunity in neonatal claves. The behavior analysis neonatal calves from of seasonal UV heat stressed dam compared to winter UV levels showed greater activity and less lying by calves born in winter during the first 2 weeks after birth (IN-HI). Cows had diverse microbial populations and greater bacterial counts on the side and udder during the summer, and the summer-born calves had greater bacteria in nasal secretions.

Sheep

It is well established that heat stress during mid- and late-gestation induces intrauterine growth retardation (IUGR) and decreased milk yield in the subsequent lactation in cattle and sheep. Members (AZ) hypothesized that elevated fetal adrenal norepinephrine (NE) secretion contributes to impaired mammary development in heat stressed pregnant ewes. Pregnant ewes were assigned to one of four treatment groups that were a combination of control (C) or hyperthermia-induced IUGR (I) and surgical sham (S) or bilateral fetal adrenal de-medullation (D) at 98 days gestational age (dGA). In the IS group, fetal plasma NE was 4-fold higher and placental, fetal and maternal mammary weights and mammary DNA were lower) compared to the three other groups that were equivalent. The number of alveolar Units per field were lower in IS and ID compared to CS and CD groups. They concluded that heat stress during gestation reduces placental, fetal and mammary development and fetal adrenal de-medullation partially reverses these effects. They also proposed that an endocrine signal from the placenta regulates maternal mammary growth during gestation and this signal is responsive to fetal catecholamines. Collaborators (AZ) also demonstrated that serotonin (5-HT) receptors 5-HT 1b, 2b, 4, and 7 are found in whole skin, apocrine sweat glands, and epithelial cells, while 5-HT 2a is present in whole skin and epithelial cells. For the 5-HT system, brain was used as a control and additional isoforms classified were 5-HT 1a, 1d, 1f, 5a,

and 6. We identified isoforms 5-HT 1d, 1f, and 5a in whole skin, isolated apocrine sweat glands, and epithelial cells, while 5-HT 1a is present in whole skin and epithelial cells, and 5-HT6 is present in whole skin and isolated apocrine sweat glands. For the PRL system, bovine mammary epithelial cells (BMEC) were the control and two isoforms were identified: PRL receptor long (PRLr-L) and PRL receptor short (PRLr-S) which differ in their intracellular domain length and sequence. They found that PRLr-S is in whole skin, apocrine sweat glands, and epithelial cells, while PRLr-L is found in only whole skin. For the ADR receptors, BMEC and liver were used as control, and researchers found Beta-1 ADR 1a, Beta=2 ADR, 1 ADR, and 2 ADR receptors in whole skin, apocrine sweat glands, and epithelial cells. Beta 1 ADR 1b was only present in BMEC and liver, while Beta 1 ADR 1d was only in BMECs. The presence of these receptors in the apocrine glands and epithelial cells indicates that the 5-HT, PRL, and ADR systems are involved in regulation of apocrine sweat gland function. This work delineated how to isolate bovine sweat glands for study and will provide the basis for further work in identifying factors that regulate sweat gland function in cattle.

Aquaculture

Near infrared reflectance spectroscopy (NIRS) was developed for both in-situ and ex-situ studies of animal physiology, nutrition and health; specifically that address questions about animal behavior, demography, reproduction or disease of Chinese Giant Salamanders. The rapid data collection, minimal sample preparation, and immediate results afforded by NIRS after calibration make it an especially attractive technology for field work within the animal management environment. The initial spectral analysis we have presented demonstrates that physiologically distinct information can be obtained from separate locations on the giant salamander's body using a surface contact NIRS field probe with only gentle drying of excessive water before scanning. Researchers currently are waiting for these animals to reach sexual maturity so that they can compare the spectral profiles from different body regions and their ability to accurately characterize gender in this species. These results are the first steps in building a useful calibration library for sex determination in the giant salamander and may also hold valuable information on skin properties from disease-free animals compared to symptomatic animals suffering from, for example, Ranavirus.

Statistical modeling

Data generated from many experiment stations (CA, MO, AZ, NE) were used to improve statistical analyses in cattle heat stress measures and heat stress abatement. Data from environmental measures were fit to a functional data analysis and hysteresis loops. The objective was to compare effects at specific times of the day (functional data analysis) vs changes over the entire day (hysteresis loops). Functional data analysis improved the ability to detect differences between cows with and without access to showers (CA) with body temperature increasing during the mid-morning. Next, hysteresis using several methods of fitting data were evaluated. Above a certain

threshold, an animals body temperature (Tb) appeared to be driven by the hot cyclic air temperature (Ta) and hysteresis occurs. The hysteresis loop showed a rotated elliptical pattern which depends on the lag between Tb and Ta.

Data from a heat stressed steer in a field experiment was next used to develop software for modeling rate-dependent hysteretic processes in farm animals with a user-friendly interface. The hysteresis package provides estimates for derived parameters along with standard errors obtained through either the delta method or bootstrapping. **H**ysteresis software provides researchers ready access to analytical tools for quantifying thermal characteristics of heat stress such as retention, cohesion and heat load, especially for animals in controlled environments and is freely available in the R software environment.

The new software shows a far greater ability to predict changes in body temperature due to the environmental heat load than alternative measures from the functional data analysis model (FDA). The FDA approach can be used to find differences in body temperature for cow-showers at specific times of day; elliptical hysteresis provides parameter estimates that act as daily summaries. Both methods provide measures that give important indicators of heat stress.

The above statistical analyses were also used for USDA-ARS and IN. The objective of this research project was to evaluate the feasibility of using the already proven hysteresis evaluation on large data sets with 12-h hourly smaller data sets. Ten cows were subjected to increased temperatures for 7 days during late gestation. Room temperature, humidity, rectal temperatures, and respiration rates of the cows were determined. Mixed models analysis showed greater respiration rates, rectal temperatures, and room temperatures during the heat treatment. Hysteresis analysis also demonstrated the treatment differences (MO). Ruminants undergo various physiological adaptations to cope with dehydration. Since the physiological mechanisms which enable ruminants to deal with water scarcity are only partly understood, a series of studies were conducted to quantify the impact of dehydration under thermoneutral and heat stress conditions. Two trials were conducted using eight Angus steers. In Trial 1, animals were dehydrated and rehydrated over 6 days under TN conditions. Trial 2 consisted of 5 days of cyclic heat stress followed by dehydration and rehydration as in Trial 1. Dehydration-induced reductions in feed intake, body weight, and respiration rate were similar in magnitude regardless of ambient temperature. Skin temperatures showed minimal change during dehydration, with an immediate drop during rehydration at both temperatures. Sweat rate declined from 100 g/m2h during heat stress to 30 g/m2h with dehydration, with no recovery until after 3 days rehydration. In both environments, rectal temperature exhibited no significant change with dehydration. Likewise, hematocrits were highly variable, decreasing during the first 24h, before returning to baseline afterwards. Serum osmolarity increased within 24h without water, and remained high until rehydration.

Accomplishments for Objective 2: Identify and assess genetic components of animal stress and well-being

Multiple studies were conducted under this objective. Cattle, pigs, sheep, and chickens were all evaluated in various experiments.

Cattle

Senepol cattle are well adapted to the tropical environment therefore, three studies were used in determining what physiological traits contribute to their adaptation. The objective of the first study (UVI, HI, NY, AZ) was to evaluate the body temperature and sweating rate of Senepol cows in the tropics. Mean temperature, relative humidity and THI during the data collection were 28.3 C, 83.7 % and 80.6, respectively. All body temperature measures, respiration rate and evaporative heat loss (EVHL) were higher among cattle in the sun than cattle in the shade. Pregnant cows had lower rectal temperature than open cows. Pregnant cows had higher vaginal temperature than open cows at all times of the day except during the morning (0600 to 1200 h). Because Senepol cattle are well adapted to the tropical environment but other Bos taurus breeds are not as well suited for the climate, the objective of the second study (UVI, HI, NY, AZ) was to compare the body temperature and sweating rate of Senepol and Charolais x Angus x Senepol heifers. Senepol heifers had lower vaginal and rectal temperature than crossbred heifers. Shaded-Senepol heifers had lower vaginal temperature than the crossbred heifers, regardless if crossbreds were shaded or not. The infrared thermography (IRR) of Senepol heifers was lower than crossbreds. For all cattle, d IRR was higher in the sun than in the shade. Senepol heifers had higher sweating rates (SWR) than crossbreds. The higher SWR of SEN heifers may influence vaginal and rectal temperatures and adaptation to the tropical climate.

This group (UVI, HI, NY, AZ) also evaluated two different methods of measuring evaporative cooling (sweating by using the portable caloric meter (Gebremedhin et al 2008 vs Vapo Meter (Delfin Technology Ltd., Kuopio, Finland) in shade and no shade environment. In addition, they compared the pregnancy status: open vs pregnant for the Senepol cattle. In cows, greater sweating rates were observed under the sun vs in the shade using the portable caloric meter. However measurements for sweating rates were lower using the Vapo Meter (<40g/m2/h) and the highest readings were observed when data were collected in PM undershade. The Vapo Meter is a closed chamber system that does not allow for radiation exposure. Pregnant cows had higher vaginal temperature vs open cows. In the heifer study, Senepol heifers had higher sweating rates than Senepol-cross breds (~7.5g/m2 vs ~20.0g/m2). Senepol heifers also had lower vaginal temperature (~0.30C) compared to the cross-breds.

The differential acute phase immune responses by Angus and Romosinuano steers were evaluated by FL, MO, and USDA-TX following an endotoxin challenge. In addition to traditional measures of heat stress, plasma concentrations of inflammatory cytokines and acute phase proteins were

measured. Results show that there are regional differences in thermal response to LPS injection, with rectal temperature providing the greater separation across breed and environment.

Sheep

Hair sheep in a tropical environments (UVI, HI, NY, and AZ) were evaluated for body temperature and sweating rate (Croix White = STX; Dorper x St Croix White = crossbred). Mean temperature, relative humidity, THI and solar radiation during the data collection were 27.4 °C, 84 %, 79.1 and 232 W/m2, respectively. Surface temperature of shaved-hide was higher in STX than in DRPX ewes. Unshaved surface temperature was 35.2 °C for both breeds. Surface temperature of shaved-hide was higher than unshaved-hide for both STX and crossbred ewes. Ewes provided shade had less respiration rates and less unshaved- and shaved-hide surface temperatures than the ewes without shade. Eye temperature was higher in the sun than in the shade for crossbred ewes but there were not eye temperature differences among STX ewes. Although sweating rates were higher in unshaded-STX ewes than shaded STX ewes, was no differences between shaded- and unshaded-crossbred ewes.

Swine

Texas A&M University, OR, and TX-Tech conducted a study to determine if changes in expression of certain stress related genes in pigs could be useful indicators of long term stress. Eleven genes (LSP1, IL4R, HSP90AA1, IL10RB, SERP1, HSF2, CCRL1, TNFRSF1A, CXCR2, IL1RN, and IL12B) showed significant increases, or decreases, across 12 h of transport stress. CCRL1 showed the greatest increase in expression of all genes between the 8 h and 12 h of transport. Cortisol, T3, and T4 also responded to transport stress and recovery. The eleven genes may be useful indicators of detrimental long-term stress.

Georgia collaborators determined the effect of heat stress on carcass weight in a commercial pig population. Phenotypes included hot carcass weight records on 227,043 commercial pigs collected from 2 packing plants located in the states of NC and MO; the pigs were a cross between purebred Duroc sires and F1 Landrace x Large White dams. The pedigree file included 553,442 animals, and 60k SNP genotypes were available for 8,232 sires. Weather information was collected from airport weather stations within 100 miles distance from the finishing farms. Average temperature humidity index (THI) was calculated for a period of 70 d before each HCW was collected. Analyses were done with an animal model as either a single-trait or 2-trait model using records identified as heat stress and comfort conditions treated as separate traits. Variance components were estimated with AI restricted maximum likelihood (REML), and traditional and genomic (G) EBV were computed either with best linear unbiased prediction (BLUP) or single-step genomic BLUP (ssGBLUP). The heritability estimate for hot carcass weight in the single-trait model was 0.20. In the multiple trait model, the heritability estimate was 0.20 under comfort conditions and 0.25 under heat stress, with a genetic correlation of 0.62. Under comfort conditions and heat stress, reliabilities in traditional EBV were 0.22 and 0.14 whereas reliabilities in genomics breeding value

(GEBV) were 0.38 and 0.19, respectively. The heritability of carcass weight is higher under heat stress.

Chickens

Collaborators (VA) investigated genetic interactions with anorexia in chickensStress was associated with increased Neuro Peptide-Y (NPY), orexin receptor 2, corticotropin-releasing factor receptor 1, melanocortin receptor 3 (MC3R), and growth hormone secretagogue receptor expression and plasma CORT. In conclusion, the loss of responsiveness to exogenous NPY in stressed LWS chicks may due to the decreased and increased hypothalamic expression of agouti-related peptide and MC3R, respectively. As such they may induce an intensification of anorexigenic melanocortin signaling pathways in LWS chicks that block the orexigenic effect of exogenous NPY.

Accomplishments for Objective 3: Development if management strategies and/or tools to enhance farm animal well-being under conditions of climatic change or stressful environments.

Dairy cattle

Several approaches to the incorporation of water into the cooling strategies used for dairy cows. Cornell, Mississippi State University and the University of Hawaii-Manoa completed a study on cooling cows by wetting the udder. The study suggested udder wetting was just as effective in cooling the high production Holstein cows compared to wetting the entire back. Control cows (no wetting) had significantly higher rectal temperature, skin temperature, and respiration rates. However, rectal temperatures and respiration rates from cows wetted at the udder or the entire back did not differ. While the udder is a smaller organ, there is a large volume of blood moving in and out of this tissue, which is likely the mechanism for the cooling effect that was accomplished with less water for cooling. The research demonstrated focusing cooling interventions where there is large volume of blood flow was just as effect as larger volumes focused on the main body in cooling cows. This would translate in less water usage for dairies.

In addition to determining novel target areas for cooling interventions, studies conducted in California and New Zealand examined how both flow rate and droplet size influence cooling effectiveness for dairy cattle. To date, spraying cattle with more water reduces respiration rate and skin and body temperature more than lower flow rates, regardless of droplet size. While the physiological benefits of cooling with more water seem clear, the behavioral responses to overhead water are mixed, with cattle readily choosing it in some studies, but avoiding it in others. We examined preferences of lactating dairy cattle for 3 cooling resources: shade alone, or shaded sprinklers differing in water flow rate and droplet size. Cattle preferences were context-dependent, based both on nozzle attributes and heat load. Sprinklers provide cooling benefits when cattle are required to stand under the spray, but in production settings animals can avoid this resource if they

choose. Consequently, behavior is an important variable that moderates the cooling effectiveness of sprinklers. We found that dairy cattle preferences for sprinklers depend on both heat load and the amount of water applied, with preferences for lower water volumes more apparent. Thus, sprinklers that output lower volumes may potentially be used more readily by animals, also conserving fresh water.

As previously discussed, water is often used to manage heat stress in dairy cattle. Sprinklers are located over the feed bunk or used while cattle are waiting to be milked, however in this experiment cattle were given control over water with a cow-activated shower. Previous studies have focused on how wetting can lower body temperature or reduce respiration rates. An alternative way to investigate this management practice is to examine internal heat loads. Internal heat load can be quantified by fitting a hysteresis loop to daily field data. The hysteresis loop is formed by a phase diagram of body temperature versus environmental input. Internal heat load is the area inside the loop. The area can be estimated using a number of environmental measures. In this study, three environmental measures were considered: ambient air temperature, the temperature-humidity index and the heat-load index. The two-stage harmonic least squares method was used to estimate internal heat load. Then a Bayesian MCMC model was used to predict internal heat load using the environmental inputs and test the effectiveness of allowing shower access on internal heat load reduction. Voluntary use of a shower reduced internal heat load and the strength of this effect increased with the degree of the heat challenge.

Additionally, the influence of the amount of shade in a pasture-based dairy system on the behavior and physiology of dairy cattle was investigated in a collaborative study between California and New Zealand, which was carried out on commercial dairy farms in New Zealand with a range of natural shade. Although animals can use shade simultaneously when it exceeds 2 m^2/animal, this amount was not sufficient for efficient cooling. More animals can use the shade at the same time when the shade amount exceeds 5 m^2/animal and this amount seems to provide more effective cooling.

Along with the lactating herds, dairy calves are susceptible to the effects of heat stress. Research in Texas determined that reflective radiant hutch covers were useful in decreasing heat load on dairy calves kept in polyethylene hutches. During data collection with highest peak temperatures of 43 ± 7.5°C, reflective covers resulted in an interior hutch temperature lower than standard hutches. Interior roof temperature for the reflective hutches was reduced compared to the control. Continued testing will seek to determine the longevity and feasibility of using this approach to calf management on commercial farms. Furthermore, there is also interest in determining the effect of these materials on cold stress in the winter months.

Research in Washington sought to alleviate heat stress in dairy calves with a slightly different approach. Plastic hutches used for neonatal dairy calf rearing accumulate heat if not properly ventilated. The use of a concrete block to elevate the hutch improved air circulation, reduced

respiratory rates and lowered heat within the hutch. This simple method can be used to reduce heat stress in dairy calves.

Beyond heat stress, lameness is a major welfare and production concern for dairy production. A two-year project on dairy cattle lameness resulted in a tested method to estimate lameness on large dairy farms, provided an investigation tool to producers and veterinarians to evaluate and mitigate lameness, assessed a quick way to identify lame cows at times when cattle are being managed for other reasons, and estimated the prevalence of lameness on farms in the Pacific Northwest. This was accomplished by modeling four methods of estimating prevalence on volunteer dairy farms and found that a sample distributed across the farms could estimate lameness prevalence within about 5 percentage points of the true prevalence. An estimate of lameness prevalence can be used to motivate investigation into causes and subsequent mitigation.

Objectives:

1. Identify measures of animal stress and well-being and characterize factors affecting the biology of stress and immune responses.

2. Identify and assess genetic components of animal stress and well-being.

3. Development of management strategies and/or tools to enhance farm animal well-being under conditions of climatic change or other stressful environments.

Methods:

Objectives will be addressed by working together to 1) develop novel technologies and biomarkers to understand the impact of stress on livestock production 2) plan and coordination research projects between project members; 3) share resources and equipment; 4) pool data and data analyses, and; 5) perform multistate summarizations of findings. Multiple projects will be initiated or continued beginning in 2017. Many projects will require multiple years to complete the investigations because of the complexity of the designs. Genetics is embedded in climate change and helping animals become resilient to stressors. The following are projected projects, however, we expect our group to expand as new scholars of stress physiology gain positions at the various universities and agencies.

Objectives: 1,3 States: WI, KS Animal: swine

Climatic changes have resulted in heat stress impacts to swine at all phase of production. Sperm production in males is particularly sensitive to even a few days of temperatures above the thermoneutral zone. In boars, temperatures above 22.7°C result in decrease semen quality beginning 2 – 3 weeks post heat event and can continue up to 8 weeks following a return to below 22.7°C. Two approaches will be used to study the problem and involve either insulation of the scrotum of boars to induce a 1.9 to 2.3°C change to testicular temperatures or to use boars in boar

studs under condition of summer heat stress with cooling approaches to the scrotum in place such as scrotal drips or whole facility cooling such as cool-cell technology. We will examine how heat stress impacts the testis and behavior of boars along with how successful heat mitigation methods are in preventing problems with semen quality in boar's of current genetics. Group members (WI, KS) will work together to identify measures of heat stress and if current mitigation methods are effective or not.

Objectives: 1,2,3 States: NY, AZ, HI, VI Animal: cattle

The consequences of thermal stress, especially on high-producing cows, are well documented. Further analysis by multiple members will focus on amelioration of heat stress, particularly for cattle. The NY collaborators will conduct economic analysis of conductive cooling compared to current practices of cooling. During thermal stress, cows will be cooled using fans, misting, and fogging. This study will look at water consumption, energy consumption, and capital costs between the 2 systems (conductive cooling and current practices). With recent progress, AZ, HI, and VI continue to investigate regulation of sweat gland function in cattle. Evaporative cooling (sweating) is the major heat alleviation mechanism in time of heat stress. During heat stress, animals increase diaphoretic response to evoke higher levels of evaporative cooling. However, except by external stimuli calling for more sweat, only a certain number of sweat glands are actively producing sweat at any given time. To increase evaporative cooling it may be possible to: (a) increase the frequency of sweating because cows sweat in a cyclic fashion, (b) stimulate sweating glands to increase sweat output, or (c) make the redundant sweat glands to sweat. To increase sweating, diaphoretic agents that stimulate sweating will be administered to dairy cows housed in the same controlled and stressful environment. Six cows, 3 control and 3 experimental, will be used to measure sweating rates. Diaphoretic agents will be administered to the 3 experimental cows. Sweating rates will be measured for 3 weeks after the cows are adjusted to the new environment for a week. Exposure to heat stress raises internal body temperature and respiration rate. To confirm, core temperature, skin surface temperature and respiration rate will be measured for each cow. The different expertise will work together to modify or enhance the natural process of sweating, and share animal and technology resources to conduct the experiments, and bring about new insight in evaporative cooling.

Objectives:1, 3 States: MT, GA, IN Animal: cattle

Not only heat stress, a consequence problem of climate change, but sudden swings in temperatures may result in cold stress particularly in free range cattle. Methods are being developed (MT and GA) to evaluate how different cattle breeds adapt to those temperature changes. GA has developed a chamber to assess those abilities. This aspect of the weaning stressors will be investigated by GA, MN, and IN and will include some genetic investigation related to the social structures.

Objectives: 1,2 States: AZ, TX, TN Animal: cattle

Methods to reduce stress in cattle are under investigation by AZ, TX, and TN. We will investigate the effect of probiotics on adrenal function during stress in cattle. Others in the group are working together to define possible antibiotic alternatives for pigs (MN and IN). With the advent of removal of antibiotics in all feeds, it becomes particularly critical to determine the best defenses.

Those antibiotic alternatives may include probiotics and associated stress responses, particularly at period of high stress such as weaning. Social competition is one of the biggest stressors following weaning because of mixing of litters at that time. This aspect of the weaning stressors will be investigated by GA, MN, and IN and will include some genetic investigation related to the social structures.

Objectives: 1 States: CO Animal: Cattle

Deficiencies and or imbalances in trace minerals can alter enzyme activity thus impairing specific metabolic pathways and ultimately, immune function and stress resilience. However, little is understood about trace mineral absorption in beef cattle. Therefore, the objectives of these experiments are to examine trace mineral transporters in ruminal epithelium and duodenal enterocytes in vivo in the presence or absence of know dietary trace mineral antagonists (Fe, Mo, and S). Eight ruminally and duodenally fistulated steers will be utilized. Steers will then be housed in individual metabolism crates and pair fed. One animal from each pair will receive supplemental Cu, Zn, and Mn at NRC (2000) concentrations. During this time, total feces and urine will be collected to calculate apparent absorption and retention values for Cu,. Zn, and Mn. At the end of the collection period, ruminal epithelial and duodenal enterocytes will be sampled and analyzed for trace mineral transporters. Similar experiments will be conducted over time with the addition of trace mineral antagonists to the diet to examine the influence of dietary antagonist on metal transporter concentrations and function.

Objectives: 1 States: KS animal: Cattle, sheep, pigs

Immunocompetence and the stress-axis are developed in the perinatal stages of life, and epigenetic changes due to the environment can program the animal's immune system, stress-axis, and behaviors. We will investigate the effect of perinatal nutrition supplementation (e.g. methionine, prebiotics) on the long term biological rhythms of behavior, immunity and stress response in dairy calves, sheep and pigs. This work will be very complimentary to the AZ, TX, TN, and CO projects, as our multistate communications will help us formulate alternatives for healthcare in livestock.

Objectives:1,3 States: NY, KS, CA Animal: cattle

Utilizing the strengths of engineers in the group (NY) and practical application (KS), methods to improve calf hutch design. Over 90% of dairy calves in the US are housed individually to reduce disease transmission. Dairy calf housing and management systems need to be dynamic to help calf resilience to climate variation. The plastic conventional calf hutch and pen systems were designed for colder seasons, but not for summer months and heat-waves. Therefore, we will modify the conventional existing plastic calf hutch to increase ventilation and cooling. Air quality, sanitation, and conductive cooling will be modified by adding a slatted-floor, waste-management system. Calves reared in the modified hutch will be compared to calves reared in conventional hutches during the summer months. Calf immune resilience and behaviors will be evaluated using novel biomarkers for stress and inflammation in the saliva and blood and automated logging devices for activity and non-nutritive oral behaviors. We will work together to engineer and re-design hutches, and share animal and technology resources to conduct the experiments.

Objectives:1,3 States: HI, IN Animal: Swine

Climate variation impacts available resources to house and manage livestock. For example, animals need bedding to thermoregulate during extreme changes in climate, but traditional bedding (eg. straw) may be a limited resource in areas of severe drought or areas where it cannot be produced. Therefore, researchers from HI and IN will implement studies of the use of compost and other bedding in free stall swine farrowing systems, focusing on measures of immune functions and adaptive behaviors.

Objectives:1 States: IN, NE Animal: cattle

IN and NE will complete data from maternal heat stress effect on offspring using a new hysteresis analysis with smaller data sets. NE, NE-USDA, and MO will evaluate heat stress using hysteresis, clustering cows based on morning and afternoon function.

Obj: 1, 2 States: VA Animal: Chicken

Chickens may undergo delayed access to feed at hatch due to processing and transportation from hatcheries. They may also be subjected to short-term cold stress during transportation. The purpose of this project is to understand the effects of delayed access to feed and cold stress on feed intake regulation, adipose tissue physiology, growth performance, and blood glucose regulation in chickens. Chickens from lines divergently selected for body weight for more than 57 generations are the model. The low-weight chickens are hypophagic and lean whereas the high-weight chickens are hyperphagic and obese. Chicks from both lines are either fed or delayed access to feed for 72 hours at hatch, and tissues collected for gene expression analysis and blood glucose measured at various time points during the first two weeks post-hatch. Chicks are also subjected to a 24 h delayed access to feed at hatch combined with a cold stress protocol to mimic transportation stress. Effects of exogenous neuropeptides on feed intake, growth, and gene expression in the hypothalamus and adipose tissue are being measured in stressed and non-stressed chickens.

Obj:1,2,3 States: NE Animal: Cattle, Sheep

β-adrenergic agonists are effective promoters of muscle growth in food animals, but the paucity of information regarding the mechanisms by which they work has caused considerable controversy and criticism. Moreover, this gap in understanding has caused many leading companies in the food industry to make high-impact decisions based solely on anecdotal evidence. Based on our preliminary studies, our overarching hypothesis is that β-agonists can be used to promote muscle growth by increasing the efficiency of muscle metabolism and myoblast function without causing direct harm to the animal. The goals of this project are to 1) provide a scientific explanation of how β-agonists affect animals at the molecular, cellular, and whole-animal levels, 2) compare the impact of supplements containing β1- or β2-specific agonists on animal performance and

wellbeing, and 3) identify mechanistic targets of β-agonists that can be used to develop better, more efficient feed additives in the future.

Obj: 1,3 States: TN, TX Animal: dairy cattle, beef cattle, swine

Food producing animals are exposed daily to environmental and management related stressors that have the capacity to disrupt homeostasis, reduce performance, and affect animal well-being. Activation of the major stress pathway, the hypothalamic-pituitary-adrenal axis, results in a significant release of the steroid, cortisol, from the cortex of the adrenal glands. Cortisol is a potent glucocorticoid with immunosuppressive effects that can lead to disease susceptibility. Corticosteroid-binding globulin (CBG) has a major influence upon the activity and availability of circulating cortisol, and thus is of relative importance in the animals' biological response to a stressor. A reduction in CBG levels, subsequent to an elevation in cortisol, can result in an increase in the free cortisol index (FCI; a surrogate measure of biologically active cortisol). Understanding physiological parameters that act as indicators of stress, such as CBG, will allow a means by which to measure responses to management practices designed to limit stress in production animals. Studies will be designed to examine circulating levels of total cortisol, CBG, FCI, and measures of behavior and immune status in swine and cattle in response to novel methods for conditioning prior to and following various management related stressors to assess their stress response.

Measurement of progress and results:

Outputs:

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The W-2173 regional research project has demonstrated a long and productive history (established in 1985). Our project members have continued to expand their collaborations over the past five years since the previous project revision. Multiple new collaborations have evolved as well as expanded accomplishments from existing collaborative teams. The outputs of these efforts are documented through clear commitment to publication of research results. Since the start of the project, our members collaborated to publish 215 peer-reviewed manuscripts and 473 other scientific papers in the form of abstracts, proceedings articles, book chapters, theses, dissertations and technical reports. Individual scientists within the group would not have been able to excel without the shared resources and the comparative, multispecies, and multi-disciplinary approach. Members of W-2173 are regular participants and/or invited speakers in special sessions and symposia on the biology of stress in livestock at national and international meetings and conferences.

Outcomes or projected impacts:

Projects directed toward the three objectives outlined in this proposal will advance the understanding of the biology of the stress response and important components and measures of animal well-being. This information will lead to improved management practices for farm animal well-being. The specific routes of information flow of these new practices to producers will include lay publications, extension publications, local and national extension meetings and conferences and national extension websites.

Projected improvements for producers may include improved: (1) methods for estimating environmental stress on animals; (2) predictions of production responses to given environmental conditions; (3) heat stress abatement methods; (4) Efficient use of energy and other resources to improve stress -resilience; (5) nutritional practices for animals exposed to environmental stress.

The application of this knowledge will reduce animal stress and increase animal productivity and reproductive potential and performance resulting in increases in net income for the livestock industry. Furthermore, the application of management strategies to alleviate stressors will improve the consumer perception image of livestock producers, especially CAFO producers and will help extensive producers improve animal well-being.

Milestones:

Year 1: Initiate multistate studies. Present findings of that first year. Re-evaluate progress and plan for further evaluation or begin manuscript preparations.

Year 2: Conduct research that furthers studies from year 1. Present findings of the second year investigations. Report published manuscripts from year 1. Plan additional collaborations based on current discoveries.

Year 3: Conduct research. Present findings of year 3 at yearly meeting. Report published manuscript from year 2. Plan additional collaborations based on previous research and new ideas. Establish committee to begin renewal project.

Year 4: Conduct research. Present findings of year 4. Report published manuscripts from year 3. Plan final year collaborations to complete studies in progress. Report planned project at the yearly meeting.

Year 5: Submit final renewal to NIMSS system. Conduct final touches to the projects.

Present findings of year 5. Report published manuscripts from year 4.

Year	Conduct research	Present findings	Publish manuscripts	Write Renewal Project
1	√	√		
2	√	√	√	
3	√	√	√	√ organize committee
4	√	√	√	√ review renewal proposal
5		√	√	submit

Projected participation: Table of resources in Appendix E.

Outreach plan:

The collaborative efforts resulting from this project are expected to continue to produce multiple peer-reviewed scientific publications, as well as abstracts of research presented at national and international meetings, non-refereed research reports, extension publications and theses/dissertations. The committee has a long history of organizing and participating in scientific symposia. It is anticipated this activity will continue. However, the primary objective of the outreach plan is to use new information to develop practical on-farm management strategies which then can be disseminated through a variety of routes. Lay and extension publications will also be produced for producer use and local, regional and national meetings such as the Southwest Nutrition Conference, The Large Herd Dairy Conference, and state production conferences will also be venues for information exchange. Additionally, several of the project participants hold extension appointments at land-grant institutions. Data and results generated from the current project that have practical application will be evaluated by extension personnel for appropriate dissemination at producer meetings.

Organization and Governance:

The recommended Standard Governance for multistate research activities will include an ex-Chair, Chair, and Secretary. Each year a Secretary will be elected and then move to the Chair and then the ex-Chair in subsequent years. Officers are to be elected for three-year terms to provide continuity. The Secretary will have the responsibility for preparation of the annual report following approval of the entire committee. Administrative guidance will be provided by an assigned Administrative Advisor and a NIFA representative.

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