

**SAES-422**  
**Multistate Research Activity Accomplishment Report**

**Project Number:** S-299  
**Project Title:** Enhancing Production and Reproductive Performance of Heat-Stressed Dairy Cattle  
**Period Covered:** 01/01/01-12/31/01  
**Annual Meeting Dates:** 02/02/02-02/03/02  
**Date of This Report:** 03/25/02

**Participants: Full list at [http://rps.uvi.edu/S299/S299\\_home\\_page.html](http://rps.uvi.edu/S299/S299_home_page.html)**

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A = Administrative advisor; P = project participant; and G = guest.

**Summary of Minutes:**

The annual meeting of the S-299 multi-state project was called to order at 9:10 a.m. on Saturday, February 2 by Chairman Scott Whisnant (NC). Present were: Lynette Edwards (TN), David Morrison (LA, Administrative Advisor), Deborah Hamernik (USDA-CSREES, Technical Advisor), Joe West (GA), John Bernard (GA), Terry Smith (MS), Bob Godfrey (U. S. VI), Scott Willard (LA), Ellen Jordan (TX), and Jerry Ward (LA). Bill Graves (GA) was a guest on February 3.

The agenda for the meeting, presented by chair Scott Whisnant, was approved. Minutes from the S-299 annual meeting in Ft. Worth were presented for discussion. Minutes were amended to include Lannett Edwards on sub-objective 8.2.B. In addition, the word oocytes was added to this objective, to reflect that the sub-objective addresses both embryos and oocytes. Amended minutes were accepted by the group.

David Morrison, administrative advisor, informed the group that there are three additions to the project; Wayne Kellogg (AR), Terry Smith (MS), and Ellen Jordan (TX). There was one withdrawal, Jean Bertrand (SC). Deborah Hamernik, technical advisor, distributed a USDA-CSREES report detailing personnel, grant funding, and changes in application dates for grants.

Minutes from the summer meeting of S-299 at Indianapolis were distributed. The web site address was amended to: [http://rps.uvi.edu/S299/S299\\_home\\_page.html](http://rps.uvi.edu/S299/S299_home_page.html). Amended minutes were approved by the group. A nominating committee to select a nominee for the secretary position was appointed, and consisted of John Bernard (chair), Ellen Jordan, and Lynette Edwards.

A discussion of progress reports ensued. For procedural purposes, secretary Joe West suggested that modifications to progress reports or protocols be handled by sub-objective leaders, thus numerous modifications will not be detailed in the minutes. During discussion of sub-objective 1.a, participants agreed that cows should not receive cooling from mist or sprinkling during trials, but that fans were acceptable. John Bernard distributed a protocol for sub-objective 1.b, cooling effects on transition cows. He will send out the protocol on the listserv soon. Bob Godfrey reported that he is working on digital thermal imaging to measure heat stress effects on bulls. There was discussion of sub-objective 2.a. regarding DCAD and yeast research.

A discussion of plans for the coming year was started. For objective 1.a., GA, LA, and MS will repeat the protocol for the coming summer. Leader for this sub-objective (Joe West) will contact other states not present to determine their participation. Plans for sub-objective 1.b. focus on cooling vs. no cooling during the dry period. Sub-objective 1.c, d, e will include work determining effects of coat color, color thermography, on male fertility.

For objective 2.b., those who can generate data for studies evaluating pregnancy can sample blood for progesterone analyses, to support pregnancy data. Those who can contribute should coordinate with Scott Whisnant and Scott Willard. For objective 2.c., there is interest in evaluating air quality in the tunnel ventilation system. Participants who have other ideas regarding air quality, animal health, or other aspects should contact Terry Smith or Scott Willard.

Ellen Jordan requested input from members regarding the need to write a protocol covering cooling pond research. The cooling pond work in Texas is currently the only cooling pond work being conducted by members of S-299, but the group agreed that a protocol should be prepared for the Environmental Modification Strategies sub-objective (Objective 8.2.c.). Others can participate as opportunities develop.

In other business, the nominating committee chair (John Bernard) reported that Terry Smith was nominated for secretary for the coming year. No other nominations were made, and Terry was elected by acclamation. The group discussed the time of year and location for the next annual meeting of S-299. After extensive discussion, a motion was made to hold the 2003 annual meeting at Franklinton, LA, and time the meeting to coincide with the meeting of SAAS in Mobile, AL. The group supported the motion, and the meeting will be held on Jan. 31, Feb. 1-2 in at the LSU Ag Center facility in Franklinton. The meeting will convene on the afternoon of Friday, January 31, and will adjourn Sunday, February 2. Included in the meeting will be a tour of the new dairy facilities at the research station in Franklinton. Jerry Ward is in charge of local

arrangements. No additional business was brought before the group and the meeting was adjourned.

### **Accomplishments and Impacts:**

**Objective 1.** To further elucidate direct and indirect effects of chronic heat stress in a hot, humid climate on nutrient intake and metabolism, endocrine status, and reproductive and lactational performance of dairy cattle.

1a. Effect of environmental stressors on body temperature, nutrient intake, and performance

During the summer of 2001, a combined nutritional and environmental influence study was conducted (GA). Forty-two lactating cows were trained to Calan gates and fed control or treatment diets, which differed in dietary cation-anion difference (DCAD). Data collected included environmental data, dry matter intake (DMI), milk yield, milk composition, cow body weight (weekly), urinary electrolytes and blood levels of glucose, electrolytes, insulin, glucagon, cortisol and thyroxine (T4). Data are being analyzed.

1b. Effect of climate pre- and postpartum on intake and performance

Studies to determine the effect of climate during the prepartum period on intake and performance postpartum will be initiated this year (GA).

1c. Effect of environmental stressors on reproductive performance (male fertility)

Discussions and planning sessions have been held with 3 local dairy farmers who are willing to participate in this portion of the project. There will be some changes made to the original protocol. Due to limitations in facilities at each farm the provision of supplemental cooling for bulls will not be possible. A schedule is being established to conduct the other portions of this project. Bulls will have semen and blood collected as described initially at intervals throughout the year that represent the times of minimal and maximal heat stress. Environmental conditions will be recorded as originally described. An addition to the project will entail the use of thermal imaging equipment in cooperation with Mississippi. Bulls will have scrotal thermograms taken at specific times of the year to attempt to quantify the effect of elevated ambient temperature on testicular temperature in the Holstein bulls and compared to Senepol bulls (heat tolerant group).

1d. Effect of genetic selection on heat tolerance or intolerance

The genetic component in heat tolerance for nonreturn rate in Holsteins was estimated using an animal linear model augmented by a random regression on a temperature humidity index (THI). Data consisted of 18,059 nonreturn rates at 45, 60, and 90 days after insemination and 81,674 first parity test-day milk yields from 78 herds in Florida. The THI on the day of insemination or test-day was added to each record. Only first insemination records were used. The model for nonreturn rate included the effects of herd-year-season, age, days in milk, milk yield, THI as a covariable, regular additive effect, and random regression on THI for heat-tolerance additive effect. With a single-trait model, heritability estimates for non-return (NR) NR45, NR60, and NR90 at THI=70 for first-lactation cows were 0.006, 0.014, and 0.053, respectively. Genetic correlation between regular NR90 and heat tolerance was -0.95. A bivariate analysis for NR90 and

test-day milk production yielded a correlation between regular merit and heat tolerance for NR90 of  $-0.35$ , substantially lower than by the univariate model, indicating a bias in the univariate estimates caused by ignored selection. The regular genetic correlation between NR90 and milk yield was  $-0.41$ . Genetic correlation between heat tolerance for NR90 and heat tolerance for milk yield was  $-0.04$ , indicating the need to separate selection.

#### Coat Color

The assistance of the Cooperative Extension Service at UVI has been obtained to schedule this portion of the project. Milk production records will be obtained from the DHIA database maintained for the St Croix herds by CES and the relationship with coat color will be determined. Coat color of bulls will also be compared to scrotal thermography data. Data will be collected beginning in the spring of 2002 and continue for at least 3 years. A subset of cows at each farm on St. Croix will be used and tracked throughout their life in the herd.

#### 1e. Effect of heat stress and production level on endocrine profiles

Experimental protocols for collaborating stations have been developed (AL) for the coming year. Samples will be sent from NC and possibly others to AL for analysis.

**Objective 2.** To develop physiological, nutritional and environmental strategies to enhance milk production, reproductive performance, profitability and heat tolerance of dairy cattle in a hot, humid climate.

#### 2a. Effect of nutritional strategies to enhance intake and efficiency of nutrient utilization and improve reproduction

Data from the DCAD treatment of the experiment reported under 1a. will be included once data analysis is complete (GA). Another trial (LA) was conducted using 40 lactating cows using different DCAD and fat levels. Supplemental DCAD reduced grain intake and increased intake of a partially mixed ration consisting of silage, alfalfa hay, whole cottonseed and ryegrass haylage. Treatments had no effect on milk yield.

A study was conducted to test the effectiveness of two different approaches at preventing or alleviating the detrimental effects of heat stress in lactating dairy cattle (MS). Firstly, use of the feed additive Fibrozyme™, which is advertised as shifting rumen digestion of forages toward that of concentrates was tested for its ability to reduce the dietary heat increment. Secondly, in an effort to offset the detrimental effects of increased respiration rate on body chemistry, a mineral supplement (Potassium Carbonate) was tested for its ability to increase rumen buffering capacity. Lactating Holstein cows ( $n = 48$ ) were placed on trial for a total of 49 days. Cows were randomly assigned to four treatment groups were balanced for age, lactation number, energy corrected milk (ECM) production and lactation somatic cell count (SCC). The four treatment groups included: Cows fed a control diet (Ctrl) which included  $0.113 \text{ kg/c/d}$  of KCl to attain a DCAD of  $+25$ . Cows fed the control supplemented with  $14 \text{ g/hd/d}$  of Fibrozyme™ (FIB). Cows fed the control diet supplemented with  $0.226 \text{ kg/hd/d}$  Potassium Carbonate supplied by DCAD plus™ to attain a DCAD of  $+35$  (CD). Cows supplemented with both Potassium Carbonate

(DCAD plus<sup>TM</sup>) and Fibrozyme<sup>TM</sup> (CD+FIB). Thus, the four treatments were CTRL, FIB, CD, and CD+FIB. Treatments were arranged as a two by two factorial. Each experimental group was fed a total mixed ration formulated to meet or exceed nutritional requirements of the animal. Data are being analyzed.

2b. Effect of hormonal supplements to enhance reproductive performance and studies on the response of oocytes and embryos to heat shock.

A collaborative study was performed in the summer of 2001 between Mississippi and North Carolina to examine whether the administration of GnRH post-breeding might improve pregnancy rates and increase serum concentrations of progesterone during heat stress in dairy cattle. While heat stress can result in reduced hormone concentrations and lower pregnancy rates in dairy cattle, other studies have shown supplemental administration of GnRH after breeding can enhance serum concentrations of progesterone and in fact improve pregnancy rates. Therefore, the objective of this study was to examine whether GnRH administration following breeding (Day 5 vs. 11 post-breeding) would enhance reproductive performance in heat-stressed dairy cows. Our findings from this trial indicate that supplemental GnRH post-breeding will increase serum concentrations of progesterone and improve pregnancy rates of cows during summer heat stress. Additional studies will be conducted this summer. The implications of these data to the producer are that summer pregnancy rates may be improved with hormonal manipulations (e.g., GnRH).

A study (TN) was designed to examine nuclear status of bovine oocytes exposed to elevated temperature after resumption of meiosis. Cumulus oocyte complexes (COC) were randomly allotted to one of four treatments and cultured at 38.5 C for 24 h (Control), 41 C for 24 h (HS024), 41 C for the first 12 h of maturation (0-12 h) followed by 38.5 C for remaining 12 h (HS012), or 38.5 C for the first 12 h of maturation followed by 41 C for remaining 12 h (12-24 h; HS1224). After 24 h, matured oocytes were denuded of cumulus; number of lysed oocytes was recorded before removal from treatment groups. Proportion of oocytes with atypical (dispersed, degenerating or condensed chromatin) versus identifiable nuclear morphologies (germinal vesicle (GV), metaphase I (MI), anaphase I (AI), telophase I (TI), or metaphase II (MII)) was recorded. Exposure of oocytes to elevated temperature, after resumption of meiosis, tended to increase proportion of lysed oocytes ( $P < 0.1$ ). Lysis was greatest for oocytes exposed to 41 C for 24 h (28 versus 14, 20 and 19% for Control, HS012 and HS1224, respectively; SEM=5.6). Proportion of oocytes with atypical nuclear morphologies was similar regardless of treatment (25, 24, 10 and 19% for Control, HS024, HS012 and HS1224, respectively; SEM=5.9;  $P=0.38$ ). Of the oocytes with identifiable nuclear morphologies, exposure to elevated temperature did not alter ability of oocytes to progress to MII. Research done with in vitro produced embryos (FL) demonstrated that in vitro fresh but not in vitro vitrified embryos had superior pregnancy rates compared to artificial insemination. Addition of insulin-like growth factor I (IGF-I) to culture media and GnRH administration embryo recipients at day 11 post-estrus increased pregnancy rates in heat-stressed cows. Finally, embryos produced from Brahman oocytes were more resistant to heat stress than those from Holstein oocytes. Further research will determine if this is due to cytoplasmic or genetic effects of the oocyte.

An *in vitro* system for monitoring, in real-time, the molecular mechanisms associated with heat stress in living bovine embryos of different genotypes is being developed (MS, USVI). Embryos from Holstein, Brahman, Senepol, Holstein x Brahman and Holstein x Senepol will be obtained at specific stages of embryonic development and transfected with HSP-70-Luciferase plasmid. Heat stress will be applied to apply acute or prolonged challenges of thermal stress and changes in HSP-70 gene expression monitored. Work is progressing (MS, FL) to develop novel means for monitoring the dynamics of heat shock proteins and other relevant genes in living embryos of different genotypes before, during and after exposure to heat stress. Photonic gene reporter technologies will be adapted for the real-time monitoring of gene expression in living embryos and reproductive tissues (CL, etc.). Equipment to develop these biophotonic paradigms has been obtained, and work will begin in Summer 2002.

#### 2c. Effect of environmental modification strategies on lactational and reproductive performance

Two groups of 10 lactating Holstein cows were housed in the tunnel ventilation barn (MS) and two similar groups were housed in adjacent freestall barns cooled with sprinklers and fans. Cows were randomly assigned to groups, and groups were balanced for production, parity, and DIM. The 10-week study began June 25, 2001 and was completed August 31, 2001. During the study, daily minimum and maximum environmental temperatures averaged  $20.7 \pm 2.2^{\circ}\text{C}$  and  $32.4 \pm 1.9^{\circ}\text{C}$ , respectively. Feed intake, refusals and milk production were recorded daily during the study. Rectal temperatures, respiration rates, and infrared surface temperatures were collected three times weekly between 1400 and 1600. The study showed that tunnel ventilation cooling decreased exposure to conditions of moderate heat stress from 345 min/d for cows housed in traditional freestall barns to 55 min/d for cows housed in the tunnel barn. The reduced heat exposure decreased the body temperatures ( $0.36^{\circ}\text{C}$ ) and respiration rates (10.6 breaths/min) for cows housed in the tunnel ventilation barn when compared to those of cows housed outside. Furthermore, this increment in cooling improved feed consumption within the tunnel barn an average of 2 kg/d over the 10-week study. After nine weeks on the trial, milk production for cows housed inside averaged 6 lbs/hd/d more than those housed outside. These results demonstrate that dairy barns cooled by tunnel ventilation can be used to mitigate the effects of heat stress in the southeastern US. Economic analysis is being conducted in conjunction with NC to determine the cost-effectiveness of such systems.

Another participant (TX) added this year will be examining the effect of cooling ponds as environmental modification on lactational and reproductive performance. Others will try to identify herds in their locations using this cooling method.

#### **Publications:**

##### **Papers:**

Dorado, CM, LM McCann, JA Miller, JL Edwards. 2001. Nuclear status of bovine oocytes exposed to elevated temperatures after resumption of meiosis. *Theriogenology* 55:469.

Edwards, JL, WA King, SJ Kawarsky, AD Ealy. 2001. Responsiveness of early embryos to environmental insults: Potential protective roles of HSP70 and glutathione. *Theriogenology* 55:209-224.

Ravagnolo, O, I Miztal. 2002. Effect of heat stress on nonreturn rate in Holsteins: Fixed-model analysis. *J Dairy Sci*: In Press.

Ravagnolo, O, I Miztal. 2002. Effect of heat stress on nonreturn rate in Holsteins: Genetic analysis. *J Dairy Sci*: In Press.

Ravagnolo, O, I Miztal. 2002. Studies on genetics of heat tolerance in dairy cattle with reduced weather information via cluster analysis. *J Dairy Sci*: In Press.

West, JW, BG Mullinix, JK Bernard. 2002. Effect of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. *J Dairy Sci*: In press.

**Abstracts:**

Al-Khatani, YM, M Drost, RL Monson, JJ Rutledge, CE Krininger III, J Block, WW Thatcher, PJ Hansen. 2002. Pregnancy rates following times embryo transfer with fresh or vitrified in vitro produced embryos in lactating dairy cows under heat stress conditions. ASAS/ADSA Meetings 2002.

Block, J, CC Chase, PJ Hansen. 2002. Inheritance of resistance of bovine preimplantation embryos to heat shock: Relative importance of maternal versus paternal contribution. ASAS/ADSA Meetings 2002.

Block, J, M Drost, RL Monson, JJ Rutledge, RM Rivera, FF Paula-Lopes, OM Ocon, PJ Hansen. 2002. Use of insulin-like growth factor-1 in culture and administration of GnRH to recipients to improve pregnancy rates following timed embryo transfer of in vitro-produced embryos to lactating dairy cows. ASAS/ADSA Meetings 2002.

Gandy, S., S. Bowers, K. Graves, A. Elias, S. Willard and C. Whisnant. 2002. Administration of GnRH post-breeding improves pregnancy rates and increases serum concentrations of progesterone during heat stress in dairy cattle. ASAS Southern Section Meetings, Orlando, FL.

Smith, T.R., S. Willard, A. Chapa, T. Riley and D. Pogue. 2002. The impact of tunnel ventilation on heat stress in lactating dairy cows: Effects on body temperature and respiration rate. ASAS Southern Section Meetings, Orlando, FL.

Spain, J.N., J. Sampson, D. Spiers. 2001. The relationship of indicators of thermal balance and milk production of cows on Missouri dairy farms. *J. Anim. Sci.* 79: Suppl. 1 p. 99.