CHEMICAL AND MANAGEMENT STRATEGIES TO CONTROL TEXAS LIVESTOCK LOSSES CAUSED BY FIRE ANTS

FINAL REPORT

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ABSTRACT

Objects of this project were (1) evaluation of livestock feeds for their ability to recruit red imported fire ants (RIFA), (2) development of an enzyme linked immunosorbent assay (ELISA) specific the RIFA venom to measure immune response in cattle and sheep, (3) evaluation of cattle breeds for immune responses to RIFA, (4) study of RIFA passive immunity transfer of cows and ewes to their offspring, and (5) study of RIFA distribution in pastures and its influence on grazing behavior in cattle. Of the six feeds evaluated, the ones with higher moisture content or higher fat content had higher attraction of RIFA; however, large pasture x feed interactions were observed. An ELISA assay to measure to antibodies (IgG) specific to RIFA venom was developed for use in cattle and sheep. Significant breed differences and family differences were observed for immune response to RIFA in a large data set of Texas cattle. Inheritance of passive immunity from pregnant cows of two breeds and ewes of two breeds was verified in young calves and lambs. Apparent genetic variation we observed in cattle would allow for selection of increased resistance to RIFA, if desired. Eight GPS tracking harnesses were constructed to monitor pasture utilization of cattle in RIFA infested areas. Due to electronic problems, these data could not be collected before August 31, 1999.

INTRODUCTION

In the Gulf Coast region of the United States the livestock industry as a whole has been economically impacted due to the presence of the red imported fire ant (Vinson, 1997). Fire ants are attracted to moist surfaces such as mucosal membranes, placental membranes, and the eyes of mammals, reptiles, and birds (TAMU, 1997). Animals that cannot move to get away from the fire ants are most susceptible to attacks. Old, weak, crippled, and very young animals fit this description, and are the most common victims of fire ant attacks (Huriash, 1997). Deer fawns, which by instinct remain still when attacked, are commonly blinded or killed by fire ants. Young calves that are born near ant mounds also suffer the same fate (Drees and Barr, 1997). Fire ant stings around the nostrils cause swelling, making breathing difficult, this results in the animal breathing through its mouth and inhaling more ants which continue to sting, leading to suffocation (Huriash, 1997). Due to the ant's attraction to moist tissue, the eyes are another common for fire ants (TAMU, 1997). Reports of deer fawns, lambs, and calves being blinded by ant stings are quite common in the southeast. Although specific effects of fire ants on grazing livestock are not well known, Texas A&M University scientists have estimated that the Texas cattle industry may loose up to $67 million per year due to fire ant related problems.

Although fire ant related allergic reactions have been a major concern in the medical field for over 50 years (Rhoades et al., 1989), allergic response to red imported fire ant venom (RIFAV), and other associated effects of fire ant stings, have not been well documented in the livestock species. Based on this knowledge, our project consisted of five distinct components. These were: (1) evaluation of livestock feeds for their ability to recruit red imported fire ants (RIFA), (2) development of an enzyme linked immunosorbent assay (ELISA) specific the RIFA venom to measure immune response in cattle and sheep, (3) evaluation of cattle breeds for immune responses to RIFA, (4) study of RIFA passive immunity transfer of cows and ewes to their offspring, and (5) study of RIFA distribution in pastures and its influence on grazing behavior in cattle. This report follows this sequence in discussion of materials and methods, results and summary.

MATERIALS AND METHODS

A. Evaluation of livestock feeds for their ability to recruit fire ants.

The objective of this study was to evaluate recruitment of red imported fire ants (RIFA) to various livestock feeds. Recruitment was investigated in three areas of Texas with one site in Taylor, Smith and McLennan counties. Feeds evaluated were extruded whole cottonseed (EWC), cottonseed meal, a horse and mule ration, a cow range cube, a calf creep ration and a commercial cat food (CAT) previously used by researchers for RIFA recruitment. One-g samples of each feed were placed into plastic bait cups. At each location bait cups were placed in a 6 x 6 latin square arrangement with two cups of each feed placed on their sides. Bait cups were recovered after 15 and 30 minutes. After all cups were collected, each site was evaluated for ant mound density and activity by survey of three 10 ft by 100 ft transects for ant mound count and rating. To approximate a normal distribution, the log of ant count per cup was the dependent variable. Data were analyzed through analyses of variance where feed, pasture, feed x pasture, row within pasture and column within pasture were independent variables. Row within pasture was used as the error term to test pasture differences.

B. Development of an enzyme-linked immunosorbent assay (ELISA) specific to venom of the red imported fire ant.

Typically, venom of other ants and stinging insects is primarily of proteinaceous origin, however, red imported fire ant venom is comprised of approximately 4-5% protein and 95% alkaloid (Ebeling, 1978; Merck, 1996). The alkaloid portion exhibits some hemolytic, cytolytic, antimicrobial, and insecticidal functions (Ebeling, 1978). The small aqueous protein fraction is most likely responsible for the allergic response, due to the fact that venom alkaloids are not know to induce allergic reactions (Merck, 1996). The venom of the fire ant serves its function as both a defense mechanism and as a means of killing prey very effectively. This venom is an extremely potent insecticide, bactericide, and fungicide (TAES, 1992).

Immunoglobulin G (IgG) is made and secreted by plasma cells in bone marrow, lymph nodes, and the spleen (Tizard, 1996). IgG is the immunoglobulin (Ig) which constitutes the greatest concentration of blood, and it is for this reason that IgG functions as the primary antibody-

mediated defense mechanism. Animals with higher levels of IgG against a specific antigen tend to have a greater tolerance to that particular substance. IgG has a fairly long half-life of about 20 days. IgG is the smallest Ig, which enables it to readily escape from blood vessels. This is extremely important in dealing with inflammation, allowing IgG to permeate tissues in order to defend body fluids and surfaces. IgG readily binds to foreign antigens and where it will clump on the surface of an antigen and initiate opsonization (Tizard, 1996).

IgG is divided into two subclasses in cattle; IgG1 and IgG2. IgG1 comprises approximately 50% of serum IgG, and is the major immunoglobulin component found in cow's milk. However, IgG2 levels are highly heritable and concentrations vary greatly between different lines of cattle. Sheep have very similar immunoglobulin subclasses to cattle, but sheep also possess an IgG3 (Tizard, 1996).

We collaborated with Dr. Laurel J. Gershwin at the University of California Vet School for this project component. For the initial development of the bovine IgG and IgE enzyme linked immunosorbent assay (ELISA), blood samples were obtained from 23 mature cows of various breed types from a fire ant endemic area, and from 5 purebred Angus cows which had never been exposed to fire ants. Blood samples were collected via coccygeal vein using 10 mL syringes and placed on ice. Blood samples were allowed to clot and centrifuged at 4500 rpm for 45 minutes so that the serum fraction could be extracted for testing purposes. Serum samples were then divided into 1.0 mL allocates and frozen for storage.

Experiment 1. The ELISA procedure was derived based on a BRSV-IgE specific ELISA procedure by Gershwin et al. (1991). For the first test, serum samples from two cows from the fire ant endemic area were compared to fetal bovine serum (FBS) and phosphate buffered saline (PBS). The assay was performed using Falcon 96 well assay plates (Fisher Scientific, Pittsburgh, PA). The test wells were sensitized with 100 μL/well of purified fire ant venom (Vespa Laboratories, Inc., Spring Mills, PA) diluted in Carb-bicarbonate buffer (pH 9.6), and incubated at room temperature for one hour. The plates were divided into two sections longitudinally with one half receiving an antigen concentration of 1 μg/well and the other 5 μg/well. The wells were then blocked using 100 μL/well of a 1% rabbit serum albumin (RSA) suspension, and then incubated one hour at room temperature. The antigen and RSA solution were then discarded, and the plates were allowed to soak for 10 minutes in PBS Tween-20 and washed six times for 2 minutes each with PBS Tween-20. The test sera were then added in a series of dilutions at concentrations of 1:10, 1:100, and 1:500, diluted in PBS. As controls, FBS and PBS were also run concurrently with the test samples.

All samples were run in duplicates of 100 μL/well and averaged together to decrease the possible effects of technician error. After samples were allowed to incubate for one hour, any non-reacted serum was removed and the plates were washed with PBS Tween-20 in the same manner as previously described. As a conjugate 100 μL/well of horseradish-peroxidase labeled rabbit anti-bovine IgG-heavy and light chain (Bethyl Laboratories, Inc., Montgomery, TX) diluted in PBS were added to the wells. The plates were laterally divided into two sections with one half receiving a conjugate concentration of 1:10,000 and the other 1:40,000. After the plates had been incubated at room temperature for one hour the unreacted conjugate was discarded and plates

were washed using the previously described procedure, with the exception that the final 2-minute wash was preformed with PBS without Tween-20. The color reaction was then initiated by adding 200 µL/well of substrate developed by combining 15 mL of citrate buffer (pH 4.5), 15 mg of o-phenylenediamine, and 6 µL of hydrogen peroxide. Plates were read with a microtiter plate reader at 492 nm after incubating for 25 minutes at room temperature.

Experiment 2. The next step was to determine if cattle from a fire ant infested area could be differentiated from cattle which had never been exposed to fire ants. Test wells were sensitized with 100 µL/well of purified fire ant venom at a concentration of 1 µg/well diluted in Carb-bicarbonate buffer pH 9.6, and incubated at room temperature for one hour. The wells were then blocked using 100 µL/well of a 1% rabbit serum albumin (RSA) suspension, and then incubated one hour at room temperature. The antigen and RSA solution were discarded, and the plates were allowed to soak for 10 minutes in PBS Tween-20 and washed six times for 2 minutes each with PBS Tween-20. One hundred µL/well of test sera were added at a concentration of 1:10 diluted in PBS. As negative controls, FBS and PBS were also run concurrently with the test samples on all plates.

All samples were run in duplicates and averaged together in order to decrease any possible effects of technician error. After samples were allowed to incubate for one hour any non-reacted serum was removed and the plates were washed with PBS Tween-20 in the same manner as previously described. As a conjugate 100 µL/well of horseradish-peroxidase labeled rabbit anti-bovine IgG-heavy and light chain diluted in PBS was added to the wells at a concentration of 1:40,000 diluted in PBS. After the plates had been incubated at room temperature for one hour the non-reacted conjugate was discarded, and plates were washed using the previously described procedure, with the exception that the final 2-minute wash was performed with PBS without Tween-20. The color reaction was then initiated by adding 200 µL/well of substrate developed by combining 15 mL of citrate buffer (pH 4.5), 15 mg of o-phenylenediamine, and 6 µL of hydrogen peroxide. Plates were read with a microtiter plate reader at 492 nm after incubating for 25 minutes at room temperature.

Experiment 3. The BRSV-IgE specific ELISA by Gershwin et al. (1991) was again used as the model in the development of a red imported fire ant venom specific bovine IgE ELISA. For the first IgE ELISA, serum samples from two cows from the fire ant endemic area were compared to one cow with no fire ant exposure, fetal bovine serum (FBS), and phosphate buffered saline (PBS). The test wells were sensitized with 100 µL/well of purified fire ant venom at a concentration of 1 µg/well, diluted in Carb-bicarbonate buffer (pH 9.6), and incubated at room temperature for one hour. The wells were blocked using 100 µL/well of a 1% rabbit serum albumin (RSA) suspension and incubated one hour at room temperature. The antigen and RSA solution were discarded, and the plates were allowed to soak for 10 minutes in PBS Tween-20 and washed six times for 2 minutes each with PBS Tween-20. The test sera were added in a series including undiluted and at concentrations of 1:2 and 1:4, diluted in PBS. As controls, FBS and PBS were also run concurrently with the test samples. All samples were run in duplicates of 100 µL/well and averaged together to decrease any potential technician error. After samples were allowed to incubate for one hour any non-reacted serum was removed and the plates were washed with PBS Tween-20 in the same manner as previously described. The primary antibody, E5, was

then added diluted in PBS at a concentration of 1:750. After being allowed to incubate one hour at room temperature, the primary antibody was removed and plates were washed following the before mentioned procedure. As a conjugate, 100 µL/well of horseradish-peroxidase labeled goat anti-mouse IgG-heavy and light chain (Bethyl Laboratories, Inc., Montgomery, TX) diluted in PBS were added to the wells.

Two plates were each laterally divided into two sections. On the first plate, one half received a conjugate concentration of 1:25,000 and the other 1:50,000, while on the second plate one half received a concentration of 1:100,000 and the other 1:600,000. After the plates had been incubated at room temperature for one hour the non-reacted conjugate was discarded, and plates were washed using the previously described procedure, with the exception that the final 2-minute wash was preformed with PBS without Tween-20. The color reaction was then initiated by adding 200 µL/well of substrate developed by combining 15 mL of citrate buffer (pH 4.5), 15 mg of o-phenylenediamine, and 6 µL of hydrogen peroxide. Plates were read with a microtiter plate reader at 492 nm after incubating for 25 minutes at room temperature.

Experiment 4. For the development of an ovine IgG ELISA specific to RIFA venom, blood samples were obtained from 10 ewes with no previous fire ant exposure, nine ewes from a fire ant endemic area, and 40 gestational ewes, of various breed compositions, which had been presented with a RIFAV challenge. Blood samples were obtained from the jugular vein using 10 mL vacutainer tubes (Fisher Scientific, Pittsburgh, PA) and placed on ice. Blood samples were allowed to clot and centrifuged at 4500 rpm for 45 minutes so that the serum fraction could be extracted for testing purposes. Serum samples were then divided into 1.0 mL allocates and frozen for storage.

The protocol for the bovine IgG previously described was used as a model, with the necessary alterations made to calibrate the assay for sheep serum. The assay was performed using Falcon 96 well assay plates (Fisher Scientific, Pittsburgh, PA). Test wells were sensitized with 100 µL/well of purified fire ant venom at a concentration of 1 µg/well diluted in Carb-bicarbonate buffer (pH 9.6), and incubated at room temperature for one hour (Vespa Laboratories, Inc., Spring Mills, PA). The wells were then blocked using 100 µL/well of a 1% rabbit serum albumin (RSA) suspension, and then incubated one hour at room temperature. The antigen and RSA solution were then discarded, and the plates were allowed to soak for 10 minutes in PBS Tween-20 and washed six times for 2 minutes each with PBS Tween-20. One hundred µL/well of test sera were then added at a concentration of 1:10 diluted in PBS. As a negative control, PBS was also run concurrently with the test samples, along with a composite of sera from 10 ewes with no previous history of fire ant exposure (O-neg). As a positive control a composite of sera from nine ewes from a fire ant endemic area (O-pos) was also run on each plate. All samples were run in duplicates of 100 µL/well and averaged together to decrease the effects of possible technician error.

After samples were allowed to incubate for one hour, any non-reacted serum was removed, and the plates were washed with PBS Tween-20 in the same manner as previously described. As a conjugate 100 µL/well of horseradish-peroxidase labeled rabbit anti-ovine IgG-heavy and light chain (Bethyl Laboratories, Inc., Montgomery, TX) diluted in PBS was added to the wells. The

plates were divided into six sections laterally and the conjugate was added at concentrations of 1:10,000, 1:20,000, 1:40,000, 1:60,000, 1:80,000, and 1:100,000 to determine the optimum concentration. After the plates had been incubated at room temperature for one hour the non-reacted conjugate was discarded, and plates were washed using the previously described procedure, with the exception that the final 2-minute wash was performed with PBS without Tween-20. The color reaction was then initiated by adding 200 μL/well of substrate developed by combining 15 mL of citrate buffer (pH 4.5), 15 mg of o-phenylenediamine, and 6 μL of hydrogen peroxide. Plates were read with a microtiter plate reader at 492 nm after incubating for 25 minutes at room temperature.

C. Evaluation of cattle breeds for immune response to the red imported fire ant.

C.1. Evaluation of different dam breeds and calf breeds.

Data. For the assessment of cattle breed differences for immune response to venom of the red imported fire ant (RIFA), nine breed types of beef cattle were utilized. All of the cows and their calves used in this study were maintained at the same RIFA infested location, the Texas A&M Agriculture Experiment Station at McGregor. Blood samples (10 mL) were collected, from 166 cows and 135 calves, at weaning in the fall of 1998. The breed types of the dams examined include Angus (n = 25), Hereford (n = 49), Nellore (n = 21), Brahman x Angus F_1 (n = 12), Brahman x Hereford F_1 (n = 13), Boran x Angus F_1(n = 7), Boran x Hereford F_1 (n = 13), Tuli x Angus F_1 (n = 15), and Tuli x Hereford F_1 (n = 12). Purebred bulls of the same breed as the dam sired all calves born to Angus, Hereford, and Nellore cows. All Brahman F_1, Boran F_1, and Tuli F_1 dams were bred to a group of transgenically cloned Brangus bulls to produce the calves used in this study. All blood samples were chilled on ice until being centrifuged in order to separate the serum fraction in preparation for IgG analysis by the previously described ELISA method.

Statistical Methods. In order to compare optical density (OD) values across different ELISA plates, the same positive and negative controls were run on every plate. Therefore, the plates can all be calibrated to each other. In this experiment two such calculations were used to calibrate the plates. The calculation based on the positive control (STD) will be referred to as IGGP, which was derived by multiplying an animal's mean OD value by the ratio of the STD mean for all plates to the STD mean for the plate that particular animal's serum was run on. Subtracting an animal's OD value from the NEG for that particular plate arrived at the negative control (NEG) based calibration, IGGN. IGGP and IGGN, of the cows, were analyzed by analysis of covariance with dam breed and regression on cow age (in years) as independent variables, using the GLM procedure of SAS (1989). Calf IGGP and IGGN were also analyzed by analysis of covariance, but with dam breed, sex, regression on cow age, and regression on weaning age (days) as independent variables. Pearson correlation coefficients were calculated through SAS to measure relationships involving cow and calf immune response to RIFA and traditional performance data.

C.2. Assessment of sire breed differences of calves in same dam breed

Data. In order to estimate the effects of sire breed on immune response to RIFAV, 89 commercial Angus cows bred to Angus, Brahman, or Nellore sires and their calves were

evaluated. The 76 offspring included straight bred Angus (n = 23), Brahman x Angus F_1 (n = 29) and Nellore x Angus F_1 (n = 24) calves. All of the cattle involved in this study were located at the Texas A&M Agriculture Experiment Station at McGregor, and had been managed in the same pasture together prior to weaning. Blood samples (10 mL) were obtained via the coccygeal vein at weaning in the fall of 1998. Samples were placed on ice until the serum fraction could be separated by centrifugation, and then stored until analyzed by the previously described bovine IgG ELISA method.

Statistical Methods. Calf IGGP and IGGN were analyzed by analysis of covariance with sire breed, regression on cow age (in years), and regression on weaning age (days) as independent variables, using the GLM procedure of SAS (1999). Pearson correlation coefficients were calculated through SAS to measure relationships involving cow and calf immune response to RIFA and traditional performance data.

C.3. Assessment of individual sires within a breed

Data. Sixty-three calves sired by 11 bulls along with their dams, were studied to determine if any differences exist between sire lines, or family lines, of Brahman cattle for immune response to the red imported fire ant. The calves and cows utilized for this study were all located at the J. D. Hudgins Cattle Company, Wharton, Texas. All blood samples were taken at weaning in Fall, 1998. Samples were then prepared for analysis by the before-mentioned bovine IgG ELISA method.

Statistical Methods. Calf IGGP and IGGN were analyzed by analysis of covariance with sire, regression on cow age (in years), and regression on weaning age (days) as independent variables, using the GLM procedure of SAS (1989). Pearson correlation coefficients were calculated through SAS to measure relationships involving cow and calf immune response to RIFA and traditional performance data.

C.4. Assessment of dam breed differences in Simmental influenced cattle

Data. In order to further assess the genetic effects on immune response to the red imported fire ant, 18 Simmental and 27 Simmental x Brahman F_1 cows and their offspring were sampled. All cows and their calves were maintained at the Eagle Crest Ranch, Van, Texas. Blood samples were taken at weaning in the fall of 1998, and analyzed by the earlier-mentioned IgG ELISA protocol.

Statistical Methods. Cow and calf IGGP and IGGN were analyzed by analysis of covariance with breed of dam, regression on cow age (in years), and regression on weaning weight as independent variables, using the GLM procedure of SAS (1989). Pearson correlation coefficients were calculated through SAS to measure relationships involving cow and calf immune response to RIFA and traditional performance data.

D. Study of passive transfer of immunity to fire ant venom in cattle and sheep.

In order to assess passive immunity to red imported fire ant venom (RIFAV), 26 beef cows and heifers and 15 ewes in late gestation, with no previous exposure to fire ants were subjected to an RIFAV challenge to stimulate production of IgG and IgE against RIFAV. The challenge consisted of RIFAV suspended in a 10% alum solution. This solution was prepared by adding 50 grams of $AlKSO_4$ to 500 mL of sterile distilled water, followed by a drop-wise addition of 1 M NaOH until a pH of 8.5 was reached. At this point the solution was centrifuged at 1500 rpm for 10 minutes, after which the supernatant was poured-off, and the supernatant was washed with borate buffered saline, and the centrifugation step was repeated. The pellet was then re-suspended in 625 mL of borate buffered saline, to bring the concentration up to of 250 grams of alum in 625 mL of solution. It was estimated that one RIFA could inject approximately 120 ng of venom through six to eight stings. The desired dosage of 120 ng of venom per mL of solution was obtained by combining 250 mL of the borate buffered saline containing100 grams of alum, with 250 mL of saline and 60 μL of fire ant venom. Therefore, each 1 mL dose contained 120 ng of venom and 200 mg of alum.

Beef Cattle. The 26 head of beef cattle used in this study consisted of 16 Angus and 10 Beefmaster cows and heifers, which were maintained at the Texas Tech University Beef Center near New Deal, Texas. All females utilized in this study were in the third trimester of gestation and bred to sires of their same breed to produce purebred offspring. On February 8, 1999, each animal was given an initial injection of 1 mL of the RIFAV suspension. At this time, 10 mL of blood were also obtained from each animal via the coccygeal vein for RIFAV specific IgG and IgE analyses by the previously described ELISA methods. Blood samples were obtained again seven days later on the fifteenth of February. Then, on March 1, a booster injection of the RIFAV suspension was given in order to induce higher IgG and IgE responses and another blood sample was taken. Subsequent blood samples were obtained on March 15, 22, and 29, April 12 and 26, and May 7 for analytical purposes. As each cow calved, the calf was added to the bleeding schedule at the next bleeding date and bled each following date thereafter. Calf blood samples were obtained by jugular veinipuncture due to the size of the calves, and ease of restraint.

Sheep. The sheep used in this experiment were kept at the Texas Tech University Sheep Center near New Deal, Texas, which consisted of 11 Rambouillet ewes and 4 Romanov-Rambouillet crossbred ewes. Rambouillet ewes were either bred to Rambouillet rams to produce purebred lambs, or to Suffolk rams to produce F_1 crossbred offspring. The Romanov x Rambouillet ewes were all bred to Hampshire rams to produce .5 Hampshire, .25 Romanov, .25 Rambouillet lambs. All ewes where in their third trimester of gestation at the onset of this experiment. These ewes received the initial 1 mL injection of the RIFAV suspension on February 19, 1999. Each ewe also had a 10 mL blood sample taken from the jugular vein at this time, which was analyzed by the previously described RIFAV specific IgG and IgE ELISA methods. On March 8, the second or booster RIFAV suspension injection was given, and another blood sample was obtained from each ewe. Subsequent blood samples were obtained from each ewe on March 15, 22, and 29, April 6 and 12, and May 3. As each ewe lambed her offspring were added to the bleeding schedule at the next scheduled date and bled each following date. Lambs less than 2 days old on their first scheduled bleeding date were not bled and were carried over until the next date for their first blood sample.

Statistical Methods. In order to compare optical density values across different ELISA plates, the same positive and negative controls were run on every plate. Therefore, the plates were all be calibrated to each other. In this experiment, two such calculations were used to calibrate the plates. The calibration based on the positive control (STD) will be referred to as IGGP, which was derived by multiplying an animal's mean IgG value by the ratio of the mean STD value for all the plates to the STD mean for the plate that animals serum run on. The negative control (NEG) based calculation (IGGN) consisted of subtracting an animal's IgG mean from the negative control for that particular plate. IGGP and IGGN for both the cows and their calves were analyzed by analysis of covariance using the GLM procedure of SAS (1989), to determine if any differences existed between sampling dates and individual cows or calves for IgG titers specific to RIFAV due to the injections given. For cow analyses, individual cow and date were included as independent variables. For calf analyses, individual calf and date were the independent variables. Angus and Beefmaster cattle were analyzed separately due to unbalance in calving dates between breeds. For analyses of the ewe data, dam breed, dam within dam breed and date were the independent variables, while for the lamb analyses, lamb breed (Rambouillet or crossbred), lamb within lamb breed and date were independent variables.

E. Study of pasture fire ant density on grazing activity of cattle.

Two types of equipment were investigated: vibracorders which measure head up and down movement, and GPS (Global Positioning System) harnesses to measure animal location within pastures. The vibracorders were commercially available and were affixed to collars, but the GPS harnesses were made by us with the help of Gerry Creager of the TAMU Mapping Sciences Laboratory in College Station. Our cattle tracking system is a system comprised of five parts that include the animal units, fixed location units, DGPS (Differential GPS) base station, data receiver base station, and a personal computer. We have collaborated with Dr. David Lunt, director of the McGregor TAES for this component.

GPS Animal Tracking Unit. The animal units were fabricated as a harness to fit around the girth of the cattle. The harness itself is made of four-inch nylon webbing, that has been padded, and with two-inch nylon webbing attached to each side. An eighteen-inch (pony) girth strap completes the harness and is attached to the two-inch nylon webbing by means of the buckles on the girth straps. Affixed to the top of the harness is the GPS tracking unit and affixed to the sides of the harness are two batteries to power the GPS tracking unit.

The GPS tracking unit itself is made up of several components. A weather tight stainless steel box was fabricated to house the GPS tracking unit. The GPS tracking unit is made up of three electronic components. The heart of the GPS tracking unit is a Motorolaa GT Oncore GPS OEM printed circuit board, which through an antenna, receives signals from orbiting satellites and also receives correction data from the DGPS base station through an onboard radio transceiver and modem. Once the signals from the satellites are received, the Motorolaa GT Oncore GPS calculates the position of the animal on the earth in latitude and longitude. Correction data received from the DGPS base station is utilized for "Real Time Correction" of the position location that was calculated from the signals received from the orbiting satellites. "Real Time Correction" is performed to eliminate positional errors caused by purposeful altering of the

satellite signals by the Department of Defense to degrade the accuracy of GPS units owned by the general public.

Transfer of the data to and from the GPS unit is facilitated by two components. A PacComma PicoPacket modem transfers the data between the radio transceiver and the Motorolaa GT Oncore GPS. Data are received and transmitted by the GPS unit, through the modem, by means of a TEKK KS-1000 radio transceiver, which has a range of approximately two miles. The radio transceiver, by means of an onboard antenna, receives correction data from the DGPS base station and transmits the corrected data from the Motorolaa GT Oncore GPS to the data receiver base station.

Power is supplied to the GPS unit by two 12V, 12ah batteries. The batteries are connected in parallel to supply 12V, 24ah power to run the GPS unit for six to seven days. Each battery is encased in a sealed steel box and the batteries are attached to either side of the harness.

GPS Fixed Point Units. Two GPS units, which are the same electronically and functionally as the GPS animal tracking units, are located directly over known, precisely surveyed locations (latitude and longitude) in the pasture with the animals. These units send corrected fixed location GPS data back to the data receiver base station which logs the data on the computer. These data are utilized as an additional correction factor to improve the accuracy of the data received from the GPS animal tracking units.

DGPS Base Station. The DGPS base station is made up of several components and functions in a manner similar to the GPS animal tracking units. A DGPS printed circuit board, obtained from Tucson Amateur Packet Radio, is mated to a Motorolaa VP Oncore GPS OEM printed circuit board to which an antenna is attached. The antenna is located directly over a known, precisely surveyed location (latitude and longitude) on the earth near the animals. This known location is programmed into the GPS and the GPS utilizes this information in conjunction with signals received from the orbiting satellites to calculate a correction factor. The correction factor data are then transferred to a radio transceiver by means of a PacComma EWM-1200 modem. The TEKK KS-1000 radio transceiver takes the data received from the modem and transmits, through an outside antenna, the data to the GPS animal tracking units for "Real Time Correction" of the GPS data.

Data Receiver Base Station. The data receiver base station functions to receive corrected GPS data from the GPS animal tracking units and transfer the data to a computer which logs the data. A TEKK KS-1000 radio transceiver, through an outside antenna, receives the data from the GPS animal tracking units and the GPS fixed point units and sends the data to a modem. The modem, a PacComma PicoPacket modem, then transfers the data to a personal computer which logs the position data of each animal and fixed point on the computer hard drive via a "Terminal" program.

Pasture Mapping. Each pasture has been mapped using Timblea GeoExplorer II hand-held GPS receivers. In addition to mapping of the pastures, the pastures will be surveyed for Red Imported Fire Ant (RIFA) mound density and activity on a four by six grid established with GPS

coordinates. The RIFA mound density and activity survey will be conducted at the same time that the GPS animal tracking units are deployed on the cattle. The pastures also will be sampled for soil and sward composition at the same time that the GPS animal tracking units are deployed on the cattle.

Collected Data. Grazing activity of the cattle will be monitored with vibracorders to determine when the cattle are grazing. The GPS data has a time stamp on it and will be used to determine where and when each animal was located at a specific grazing event. All collected data will then be analyzed using mapping software on a computer to determine if the RIFA has an effect on cattle grazing in a RIFA infested pasture.

RESULTS AND DISCUSSION

A. Evaluation of livestock feeds for their ability to recruit fire ants.

Nutritional aspects of the feeds are presented in Table 1, and mean ant count per cup at 15 (AC15) and 30 minutes (AC30) are presented in Table 2. Differences were observed for feed ($P < .001$), pasture ($P < .001$) and feed x pasture ($P < .05$) for ant count after 15 (AC15) and 30 minutes (AC30). The commercial cat food (CAT) had the highest AC15 per cup in five of seven pastures (20.3 to 74.5), and extruded cottonseed (EWC) had highest AC15 in the other two (24.2 to 67.7). The EWC had the highest AC30 in six of seven pastures (69.3 to 236.2), and CAT had the highest count in one pasture (17.5). Across all pastures, CAT and EWC had similar AC15 (36.6 and 35.7, respectively) with others ranging from 3.2 to 9.5 ants per cup. For AC30, EWC averaged 114.0, CAT averaged 56.2 and the rest ranged from 1.7 to 15.8. All pastures had higher ant counts at 30 minutes versus 15 minutes except one. AC15 per pasture ranged from 6.0 to 32.2, whereas AC30 per pasture ranged from 3.8 to 57.5. Density of RIFA mounds across pastures (Table 3) ranged from 64 to 197 per ha, but did not match exactly with AC15 or AC30. Figures 1 and 2 illustrate the interactions observed between ant counts per cup at 15 and 30 minutes, respectively.

B. Development of an enzyme-linked immunosorbent assay (ELISA) specific to venom of the red imported fire ant.

Experiment 1. The results from the initial IgG ELISA plate indicated the red imported fire ant venom (RIFAV) to be a very strong antigen; therefore, it was determined that an antigen concentration of 1 μg/well would be applied. From this initial test it was also decided that serum samples would be best utilized at a concentration of 1:10 diluted with PBS. Due to the strong binding properties of the RIFAV, it was concluded that the IgG conjugate should be employed at a concentration of 1:40,000 to reduce the risk of nonspecific binding. These concentrations were selected because the resulting optical density (OD) values were deemed optimum by dividing the positive control (in this case the mean OD value of the cattle from the fire ant infested area) OD value by the negative control OD value to equate a positive:negative (P:N) ratio. The larger the P:N ration, the greater level of differentiation the test will exploit.

Experiment 2. The results of this test showed a significant difference in the optical density (OD) readings of the cows from the fire ant endemic area as opposed to those cows with no previous exposure to fire ants and the negative controls. The resulting P:N ratio remained comparable to the initial plate. From these findings the IgG ELISA protocol would be adopted for use in further research. Additionally, sera from six of the cows from the fire ant infested area were combined to form a composite serum (STD) to be used as a positive control. This composite serum would be run on all future plates along with FBS and PBS as negative controls, so that all of the plates could be calibrated to each other.

Experiment 3. With the information obtained from these two initial IgE ELISA tests and due to the low percentage of IgE typically found in bovine serum, it was concluded that the serum samples would be applied undiluted. Again, due to the low concentration of IgE present, a conjugate concentration of 1:25, 000 was necessary to capture significant amounts of serum IgE. This was determined using the P:N ratio described in the IgG protocol.

The IgE protocol was finalized as follows. Test wells were sensitized with 100 µL/well of purified fire ant venom at a concentration of 1 µg/well, diluted in Carb-bicarbonate buffer pH 9.6, and incubated at room temperature for one hour. The wells were then blocked using 100 µL/well of a 1% rabbit serum albumin (RSA) suspension, and incubated one hour at room temperature. The antigen and RSA solution were discarded, and the plates were allowed to soak for 10 minutes in PBS Tween-20 and washed six times for 2 minutes each with PBS Tween-20. One hundred µL/well of test sera were then added undiluted. The positive control STD and negative controls, FBS and PBS were also run concurrently with the test samples. All samples were run in duplicate to be averaged together to decrease any potential effects of technician error. After samples were allowed to incubate for one hour, any non-reacted serum was removed and the plates were washed with PBS Tween-20 in the same manner as previously described. The primary antibody, E5, was then added diluted in PBS at a concentration of 1:750. After being allowed to incubate one hour at room temperature, the primary antibody was removed and plates were washed following the before mentioned procedure. As a conjugate, 100 µL/well of horseradish-peroxidase labeled goat anti-mouse IgG-heavy and light chain diluted in PBS was added to the wells at a concentration of 1:25,000.

After the plates had been incubated at room temperature for one hour, the non-reacted conjugate was discarded, and plates were washed using the previously described procedure, with the exception that the final 2-minute wash was performed with PBS without Tween-20. The color reaction was then initiated by adding 200 µL/well of substrate developed by combining 15 mL of citrate buffer pH 4.5, 15 mg of o-phenylenediamine, and 6 µL of hydrogen peroxide. Plates were read with a microtiter plate reader at 492 nm after incubating for 25 minutes at room temperature. The IgE assays did not show reliable results after several modifications; therefore, IgE titers were not analyzed.

Experiment 4. Results from the initial ELISA showed the conjugate concentration of 1:40,000 diluted in PBS to give the optimum positive to negative ratio (P:N). Therefore, the following protocol was adopted for all subsequent ovine IgG assays. The test wells were sensitized with 100 µL/well of purified fire ant venom at a concentration of 1 µg/well diluted in Carb-bicarbonate

buffer pH 9.6, and incubated at room temperature for one hour. The wells were then blocked using 100 μL/well of a 1% rabbit serum albumin (RSA) suspension, and then incubated one hour at room temperature. The antigen and RSA solution were discarded, and the plates were allowed to soak for 10 minutes in PBS Tween-20 and washed six times for 2 minutes each with PBS Tween-20. One hundred μL/well of test sera were then added at a concentration of 1:10 diluted in PBS. The positive control (O-pos) and negative controls (O-neg and PBS) were also run concurrently with the test samples on all plates. All samples were run in duplicates and averaged together in order to decrease the possible effects of technician error.

After samples were allowed to incubate for one hour, any non-reacted serum was removed, and the plates were washed with PBS Tween-20 in the same manner as previously described. As a conjugate 100 μL/well of horseradish-peroxidase labeled rabbit anti-ovine IgG-heavy and light chain diluted in PBS was added to the wells at a concentration of 1:40,000 diluted in PBS. After the plates had been incubated at room temperature for one hour, the non-reacted conjugate was discarded and plates were washed using the previously described procedure, with the exception that the final 2 minute wash was preformed with PBS without Tween-20. The color reaction was then initiated by adding 200 μL/well of substrate developed by combining 15 mL of citrate buffer pH 4.5, 15 mg of o-phenylenediamine, and 6 μL of hydrogen peroxide. Plates were read with a microtiter plate reader at 492 nm after incubating for 25 minutes at room temperature.

C. Evaluation of cattle breeds for immune response to the red imported fire ant.

C.1. Evaluation of different dam breeds and calf breeds.

In this discussion, references to two IgG measures are made. The optical density among cows adjusted for the positive control per plate is referred to as IGGP, whereas the level adjusted for the negative control per plate is denoted as IGGN. These measures among calves are referred to as IGGPC and IGGNC, respectively. Across the nine breed-types of beef cattle analyzed there were significant differences for IgG antibody titers to RIFA venom. Based on the OD values of the cows, dam breed was significant for IGGP ($P < .05$) and IGGN ($P < .0005$). Regression on cow age in years was significant for cow IGGP .01 ± .003 ($P < .001$) and IGGN .009 ± .003 ($P < .001$). Means values for IGGP and IGGN across dam breeds are presented in Table 4.

Based on the calves' OD values, regression on dam age in years, regression on calf weaning age in days, and the effect of calf sex were not significant for IGGP or IGGN. Dam breed did not account for significant variation in IGGP, but was significant for calf IGGN ($P < .0002$). These mean in calves are in Table 5.

Correlations involving production measures and immune responses across all breeds are presented in Table 6. Across all cows, the correlation between IGGP and IGGN was .79 ($P < .0001$). The simple correlation between IGGPC and IGGNC, across all calves, was .52 ($P < .0001$). However, the correlation between IGGN over all the cows with that in their calves (IGGNC) was -.18 ($P < .05$). The correlation between IGGP of all the cows with that in their offspring (IGGPC) was also negative, but was not significant. It should also be noted that across all calves,

the correlation between calf IGGP and birth weight was .19 ($P < .05$). And, the correlation between IGGN and birth weight, across all calves, was .18 ($P < .05$).

In study of the correlations individually within dam breeds, some interesting and unclear observations were seen. There was a large range in degree of relationship between birth weight and weaning weight of calves with IgG measures in both the dams and the calves themselves. Part of this confusion is due to the variation in animal numbers across these dam breeds. In Nellore cows and calves, there was a correlation of .55 between both IGGPC and IGGNC and birth weight; in these calves, the correlation involving weaning weight and IGGP and IGGN was .58 and .53, respectively. However the correlation of IGGPC or IGGNC with birth weight was -.29. Other dam breeds showed similar results, but some illustrated that the dam's IgG measures were more highly correlated with birth and weaning weights in their calves than the IgG measures in the calves themselves. Still other breeds exhibited very little relationship between IgG measures and these performance traits.

The correlation between IgG measures in the cows (IGGP and IGGN) were highly correlated (.95 to .99) across all dam breeds except Angus (.38). The relationship between IgG measures in the calves (IGGPC and IGGNC) was more variable (.14 to .99 range). Furthermore, the correlation between the IgG measure based on the positive control in cows (IGGP) and calves (IGGPC) and correlation between the IgG measure based on the negative control in cows (IGGN) and calves (IGGNC) were variable, but negative in all but Angus and Boran x Angus cows.

C.2. Assessment of sire breed differences of calves in same dam breed.

Across the three breed-types of calves analyzed in this study, there were no significant effects of breed, regression on cow age, and regression on weaning age on IgG antibody titers. It should be noted that the R-square values for IGGP (.0361) and IGGN (.0547) were very low. This suggests that the sire's genetics contribute may contribute less to IgG levels observed in young calves than that of the dams. The simple correlation between IGGP and IGGN of the calves remained high at .61 ($P < .0001$).

C.3. Assessment of individual sires within a breed

Across all calves there were some significant differences for RIFAV specific IgG titers. Individual sire was significant across all calves for IGGP ($P < .0005$), but not important for IGGN. These results indicate possible genetic differences for RIFAV specific IgG within the Brahman breed. Regression on cow age was also significant for IGGP $-.0119 \pm .0058$ ($P < .05$), but insignificant for IGGN. Regression on weaning age was found to be insignificant for both IGGP and IGGN. The simple correlation between cow IGGP and cow IGGN was .32 ($P < .01$). The correlation between calf IGGP and calf IGGN was .36 ($P < .005$).

C.4. Assessment of dam breed differences in Simmental influenced cattle

Across both dam types, dam breed and regression on cow age were insignificant for IGGP. Dam breed was significant for cow IGGN ($P < .05$), but regression on cow age was insignificant. Across all calves dam breed, regression on cow age, and regression on weaning weight were insignificant for IGGP and IGGN. The correlation between cow IGGP and cow IGGN was .58 ($P < .0001$). Calf IGGP and calf IGGN showed a correlation of .63 ($P < .002$).

Overall discussion of project component C (breed differences).

Although immune responses of cattle and sheep that are specific to RIFA have not been documented before this project, several aspects involving breed effects are related to other reports. Muggli et al. (1987) studied genetic variation for serum IgG1 and antibody titers against infectious bovine rhinotracheitis virus (IBRV). This study employed a herd of three hundred sixty seven Hereford calves from four genetically selected lines, and a commingled herd comprised of 79 Angus, 40 Hereford, and 46 Red Poll calves. Blood samples were obtained between 24 and 48 hour postpartum to be analyzed for IgG1 concentration, and prior to IBRV vaccination and 60 days post-vaccination to be examined for IBRV antibody titers. Within the Hereford herd consisting of four separate genetic lines no significant differences in immune function were evident. However, within the commingled herd, Angus calves recorded higher IgG1 means than Hereford or Red Poll calves. It should also be noted that calves from older dams (>3 years of age) had higher IgG1 means and IBRV antibody titers than younger dams (Muggli et al. 1987). Engle et al. (1999) also found higher IgG levels to pig red blood cells in Angus compared to Simmental steers. Several of our results indicate that Angus cattle has different immune responses to RIFA compared to several other breeds. However, obvious trends consistent across all genetic types were not distinguishable.

Although heritability of immune response to RIFA was not formally measured in these data due to lack of family size, there was a large sire effect in Brahman cattle for IGGP measured in calves. Based on half-sib analysis, the estimated heritability would be between .60 and 1.0, but these calves are probably more highly related than just through unrelated sires, so this estimate must be viewed carefully. Brown et al. (1992) estimated the heritability of horn fly resistance for seven different breed types of beef cattle. Cows (n=215) were managed in separate breeding pastures at the same research station over a period of four years. During the horn fly season (May through November), cows were individually observed weekly and horn fly densities were recorded on a per cow basis. Those individuals with a consistently low average number of horn flies were considered to have some level of resistance, which was assumed to be a normally distributed, quantitatively inherited trait. Results from this study showed that there were differences between breeds for horn fly density. The authors estimated repeatability to be $.47 \pm .05$, and heritability to be $.78 \pm .16$ for paternal half sibs and $.59 \pm .10$ using twice the intrasire regression of daughter on dam. Therefore, if a producer were to incorporate horn fly resistance into the normal selection criterion, economic benefits could be realized (Brown et al. 1992).

Hammond et al. (1997) documented a significant sire effect for IgG titer to stomach nemotodes and stated that this genetic information could be used as a selection tool to increase resistance to these parasites. It appears that selection for increased immune response to RIFA in cattle and

sheep could also increase their resistance. The main caveat to our project is that there is no way to know how much RIFA exposure cattle of these different breeds have had. If there was a consistent trend across breeds for RIFA-specific IgG measure, the level of exposure could be predicted from the IgG measure; however, this is probably not the case when we have observed so many differences between breed types with a single location.

D. Study of passive transfer of immunity to fire ant venom in cattle and sheep.

Beef Cattle. The 120 ng of RIFAV per mL dosage appeared to induce a slight IgG response in the challenged cows and their offspring. Based on the IGGP of the Angus cows and heifers, individual cow effect was large ($P < .0001$). Individual cow least squares means for IGGP over a 9-week period ranged from .2097 to .4773. Average IgG values across times in Angus cows and calves are in Table 7. A borderline significance between bleeding dates was observed ($P = .0507$). It should be noted that among the Angus cows and heifers, IGGP values decreased slightly after the initial injection, but then increased approximately 2-weeks after the booster injection, peaked 2-weeks later and then appeared to taper off.

Aditionally, the IGGN calculations for the Angus cows and heifers also showed a strong individual cow effect ($P < .0001$). Cow IGGN means ranged from .1021 to .2319 over the study period. However, the bleeding date effect was insignificant. Though insignificant, IGGN values showed the same trend over time as those for IGGP.

The IGGP of the Angus calves exhibited similar results to their dams. Individual calf effect was very large ($P < .0001$). Calf IGGP means ranged from .3052 to 1.0930. Sampling date was of borderline significance for calf IGGP ($P = .0760$). IGGN of the Angus calves again showed individual calf effect to be important ($P < .02$). Means for calf IGGN ranged from .0956 to .1679. Bleeding date was also a highly significant source of variation ($P < .001$).

The Beefmaster cows and heifers showed similar results to the Angus. Based on IGGP, individual cow effect was quite large ($P < .0001$). Individual cow means ranged from .2693 to .5825 over the twelve-week period, which appeared slightly higher on average than the Angus cattle. Mean IgG measures in Beefmaster cows and calves across times are presented in Table 8. The IGGP means for the Beefmaster cattle also showed an increase 5-weeks after the initial injection, temporarily peaked 2-weeks later, tapered off, and then rose again on weeks 10 and 12. It should be pointed out that the Beefmaster cows were sampled twice more than the Angus in order to include more offspring, due to their later calving season.

The IGGN results for the Beefmaster cows were again similar to the Angus. The individual cow effect was again very large ($P < .0001$). The means for IGGN ranged from .1115 to .2328. Sampling date was not significant for IGGN of the Beefmaster cows and heifers. Based on the IGGP and IGGN on the Beefmaster calves, neither calf effect nor date was found to be significant. This could be attributed to the small number of Beefmaster calves ($n = 4$) in the data set.

Sheep. As with the beef cattle, some significant differences were observed within the sheep for an IgG response to RIFA venom exposure. The breed of the ewe, (dam breed), was found to be very important for both IGGP ($P < .0001$) and IGGN ($P < .0001$). The IGGP mean for the Rambouillet ewes was .2365 versus .1864 for the Romanov x Rambouillet ewes. Correspondingly, the IGGN means were .0824 and .0466, respectively for Rambouillet and Romanov x Rambouillet ewes. Dam within breed was also very large for IGGP ($P < .0001$) and IGGN ($P < .0001$) variation. Blood sampling date was also important for IGGP ($P < .0006$) and IGGN ($P < .0001$). Average IgG values across times for ewes and lambs are in Table 9.

The data obtained from the lambs showed that lamb breed, lamb within breed, and sampling date were all very large sources of variation for both IGGP ($P < .0001$) and IGGN ($P < .0001$). However, in contrast to the ewe data, the Rambouillet lambs had lower IGGP and IGGN means than the crossbred lambs. The IGGP means were .1492 for the Rambouillet lambs and .1768 for the .5 Hampshire, .25 Rambouillet, and .25 Romanov lambs, while IGGN means were .0381 and .0521. The means for both IGGP and IGGN across all lambs showed a steadily decreasing trend throughout the study period.

As far as we can determine, inheritance of passive immunity to RIFA venom has not been reported until now. Both the cattle study and the sheep study indicate that strong individual animal and breed variation in immune response to RIFA venom. All analyses were conducted with the same dosage of RIFA venom. This study also indicated that immunity of RIFA venom is passively transferred to offspring of cows and ewes exposed to RIFA venom during late gestation. Future studies with increased and varying dosages to assess possible time-dosage and breed-dosage interactions could provide additional insights into how these species respond to RIFA exposure.

E. Study of pasture fire ant density on grazing activity of cattle.

The aspect of this project that has shown some of the most promise has also shown the most disappointment. To date all equipment has been fabricated and sixteen steers have been transported to the Texas Agricultural Experiment Station at McGregor. The plan was to deploy the GPS animal tracking units on the cattle and collect data for a consecutive four-week period between the months of June and July. Upon arrival the equipment was tested and found to have operational problems. Those errors were corrected and the equipment was tested again. All of the equipment was functioning properly with the exception of the differential correction by the animal tracking units. The units have worked in gathering and transmitting data, but without the differential correction the error of position is 15 meters as opposed to approximately 1 meter. Gerry Creager has been trying to trouble shoot the problem, but to date, has not been able to determine where the problem lies. Once the differential correction problem has been rectified, the research will proceed. The cost of our units has been approximately $700 per unit, which is much cheaper than that of commercially available units.

SUMMARY

A. Evaluation of livestock feeds for their ability to recruit fire ants.

Our limited sampling of commercially available livestock feeds has shown that large differences exist in these feeds' abilities to recruit RIFA. The ants were primarily attracted to feeds that contained a large percentage of water or fat. Five livestock feeds and a commercial cat food were evaluated in three counties in Texas. Large differences between pasture-feed combinations were observed. Certainly the soil characteristics at different locations may have caused the pasture-feed interactions observed. This research should be expanded to several other feeds because supplementation practices by livestock producers could influence attraction of fire ants.

B. Development of an enzyme-linked immunosorbent assay (ELISA) specific to venom of the red imported fire ant.

The previously described ELISA procedures have served as a tool to study the effects of red imported fire ants (RIFA) on cattle and sheep and will be useful for future research. Through the development of the ELISA protocols a significant difference between serum of cattle with a history of fire ant exposure and that of unexposed cattle was noted. Therefore, the possibility of identifying genetic differences for IgG and IgE to fire ant venom has been explored. With the successful development of these ELISA protocols further research was conducted in an effort to determine if any genetic differences exist for immune response to fire ant venom, which may benefit livestock producers through genotype-environment management considerations.

C. Evaluation of cattle breeds for immune response to the red imported fire ant.

Results from this study have shown that significant breed differences exist for immune response to the red imported fire ant. The genetically diverse group of cattle analyzed during this study exhibited significantly different IgG antibody titers against RIFA, which could possibly benefit the cattle producer through management considerations. The results also imply that the breed-type of the dam may have a very large effect on calf IgG titer. In some breeds, fairly large correlations were observed between the dams' IgG titers and birth weight or weaning weight of the calves. In other breeds, fairly large correlations were seen between the calves' IgG titers and their birth weights or weaning weights. These results indicate genetic differences for RIFA venom specific IgG within the Brahman breed where the heritability for immune response (IGGP) was estimated to be between .60 and 1.0. Further research, comparing more breed types of beef cattle, is needed to fully understand the immune response to the RIFA and the negative impacts of fire ant infestation on cattle operations. Our results illustrate that enough genetic variation exists in cattle for immune response to RIFA to select for increased fire ant resistance if desired.

D. Study of passive transfer of immunity to fire ant venom in cattle and sheep.

The results indicated that the 1 mL dose of RIFA venom suspension containing 120 ng of RIFA venom protein induced a small response in both the beef cattle and the sheep evaluated in this experiment. Further research is needed to explore the possibility of presenting dams previously unexposed to RIFA with a RIFA venom challenge prior to parturition in an attempt to raise IgG titers of offspring born into RIFA endemic areas. The data have shown that there are individual animal effects that influence the degree of reaction a cow or ewe will have to the introduction of

RIFA venom. Due to some differences in sampling dates, Angus and Beefmaster cattle could not be fairly compared to each other, but appear to show different trends. For all blood collection times, there appeared to be higher IGGP values in Angus calves than in the dams when only the dams were exposed to RIFA venom.

Significant breed differences were identified for RIFAV response between the two types of ewes studied. Rambouillet ewes built higher IgG titers in response to the IFAV challenge than the Romanov x Rambouillet ewes. However, lambs reared by Rambouillet ewes had lower IgG titers to RIFA venom when their dams had been challenged prior to parturition, than those reared by the crossbred ewes. Whether this effect was due to the lambs themselves being crossbred or an effect of their dams being crossbred could not be determined from this experiment. These results indicate that more research is necessary to understand specific breed differences in both cattle and sheep and to also examine families within breeds for response to RIFA venom. This knowledge could further enable livestock producers to adapt to the RIFA problem in the southern United States through its incorporation into selection/culling programs.

E. Study of pasture fire ant density on grazing activity of cattle.

The aspect of this project that has shown some of the most promise has also shown the most disappointment. To date all equipment has been fabricated and tested; sixteen steers were been transported to the Texas Agricultural Experiment Station at McGregor to study pasture utilization related to RIFA mound distribution and fitted with units. However, due to persisting electronic problems with differential correction from fixed points, no accurate data could be obtained before the end of this two year cycle. The cost of our units has been approximately $700 compared to at least $3,000 per unit for commercially available models.

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TABLE 1. Nutritional Analyses of Livestock Feeds (As Fed Basis) Used to Measure Fire Ant Recruitment

Feed	DM[1] %	CP[2] %	ADF[3] %	Fat[4] %
Extruded whole cottonseed	94.6	21.1	39.1	19.5
Cottonseed meal	91.4	44.2	15.5	2.5
Horse and mule	89.1	11.2	15.9	4.2
Range cube	92.1	20.3	11.2	2.7
Calf creep feed	90.6	14.0	21.9	3.1
Cat food	64.5	26.5	5.3	8.0

[1]DM = dry matter
[2]CP = crude protein
[3]ADF = acid detergent fiber
[4]Fat = crude fat

TABLE 2. Mean Fire Ant Count per Cup Across Two Times

Feed	15 minutes	30 minutes
Extruded whole cottonseed	35.7[a]	116.8[a]
Cottonseed meal	9.5[b]	15.8[b]
Horse & mule	8.0[b]	11.2[c]
Range cube	3.2[c]	3.6[d]
Calf creep feed	4.1[c]	3.7[d]
Cat food	36.9[a]	56.1[a]

[a – d] Means within a column with different superscripts differ ($P < .05$), based on analyses of log transformed data.

TABLE 3. Fire Ant Mound Density in Feed Recruitment Pasture Study Sites

Location	Active mounds per hectare
Taylor Co. 1	897
Smith Co. 1	788
Smith Co. 2	1218
Smith Co. 3	825
McLennan Co. 1	610
McLennan Co. 2	395
McLennan Co. 3	610

TABLE 4. Least Squares Means and SE for IgG Immune Response to Fire Ant Venom in Cows across Breeds at McGregor TAES at Weaning in October, 1998.

Dam breed	n	IGGP[1]	IGGN[2]
Angus	25	.564 " .016	.380 " .018
Brahman x Angus	12	.575 " .022	.344 " .025
Brahman x Hereford	13	.571 " .021	.326 " .024
Hereford	49	.510 " .012	.331 " .014
Boran x Angus	7	.527 " .029	.282 " .033
Boran x Hereford	13	.547 " .021	.305 " .024
Nellore	21	.526 " .018	.382 " .020
Tuli x Angus	15	.509 " .020	.256 " .023
Tuli x Hereford	12	.560 " .022	.308 " .025

[1]IGGP = optical density reading corrected for positive control per plate.

[2]IGGN = optical density reading corrected for negative control per plate.

TABLE 5. Least Squares Means and SE for IgG Immune Response to Fire Ant Venom in Calves across Dam Breeds at McGregor TAES at Weaning in October, 1998.

Dam breed	n	IGGPC[1]	IGGNC[2]
Angus	22	.423 " .016	.194 " .021
Brahman x Angus	12	.399 " .019	.281 " .026
Brahman x Hereford	11	.431 " .020	.341 " .027
Hereford	37	.396 " .013	.228 " .017
Boran x Angus	6	.375 " .028	.245 " .036
Boran x Hereford	13	.456 " .019	.305 " .024
Nellore	9	.389 " .023	.292 " .030
Tuli x Angus	15	.403 " .018	.314 " .023
Tuli x Hereford	11	.388 " .020	.265 " .027

[1]IGGPC = optical density reading corrected for positive control per plate.

[2]IGGNC = optical density reading corrected for negative control per plate.

TABLE 6. Pearson Correlation Coefficients[1] Involving IgG Immune Response to Fire Ant Venom and Production Measures in Cows of Different Breeds and Their Calves at McGregor TAES at Weaning in 1998.

	IGGP[2]	IGGN[3]	IGGPC[4]	IGGNC[5]
COWWT[6]	-.0418	.0632	-.0854	-.0403
	.5894	.4142	.3209	.6398
COWAGE[7]	.2439	.2207	.0270	-.1384
	.0015	.0041	.7577	.1081
WAGE[8]	.2683	.1874	.0060	-.1240
	.0007	.0195	.9443	.1490
BWT[9]	-.0021	-.1351	.1932	.1810
	.9797	.0927	.0237	.0343
WWT[10]	.1035	-.0247	-.0632	.0331
	.2016	.7612	.4628	.7013
IGGP		.7921	-.1102	-.1350
		.0001	.2086	.1226
IGGN			-.1530	-.1832
			.0798	.0355
IGGPC				.5158
				.0001

[1]Correlation coefficient listed above level of significance (P-value).
[2]IGGP = optical density reading in cows corrected for positive control per plate.
[3]IGGN = optical density reading in cows corrected for negative control per plate.
[4]IGGPC = optical density reading in calves corrected for positive control per plate.
[5]IGGNC = optical density reading in calves corrected for negative control per plate.
[6]COWWT = cow weight at weaning.
[7]COWAGE = cow age in years at calf weaning.
[8]WAGE = weaning age of calf.
[9]BWT = birth weight of calf.
[10]WWT = weaning weight of calf.

TABLE 7. Least Squares Means and SE for IgG Immune Response Specific to Fire Ant Venom Across Time in Gestating Angus Cows and Their Calves

	Cows			Calves		
Date	n	IGGP[1]	IGGN[2]	n	IGGPC[3]	IGGNC[4]
Feb. 2, 1999	18	.335 " .010	.164 " .007			
Feb. 15, 1999	17	.328 " .010	.159 " .008	1	.452 " .137	.143 " .037
Mar. 1, 1999	17	.327 " .010	.159 " .008	6	.564 " .057	.159 " .015
Mar. 15, 1999	18	.353 " .010	.165 " .007	6	.593 " .057	.137 " .015
Mar. 22 1999	18	.364 " .010	.177 " .007	9	.397 " .046	.148 " .012
Mar. 29, 1999	18	.356 " .010	.156 " .007	9	.530 " .046	.116 " .012
Apr. 12, 1999	17	.341 " .010	.148 " .008	12	.497 " .036	.093 " .010

[1]IGGP = optical density reading in cows corrected for positive control per plate.
[2]IGGN = optical density reading in cows corrected for negative control per plate.
[3]IGGPC = optical density reading in calves corrected for positive control per plate.
[4]IGGNC = optical density reading in calves corrected for negative control per plate.

TABLE 8. Least Squares Means and SE for IgG Immune Response Specific to Fire Ant Venom Across Time in Gestating Beefmaster Cows and Their Calves

	Cows			Calves		
Date	n	IGGP[1]	IGGN[2]	n	IGGPC[3]	IGGNC[4]
Feb. 2, 1999	10	.344 " .025	.159 " .009			
Feb. 15, 1999	10	.355 " .025	.157 " .009			
Mar. 1, 1999	10	.346 " .025	.149 " .009			
Mar. 15, 1999	9	.377 " .026	.155 " .009			
Mar. 22 1999	9	.390 " .026	.167 " .009			
Mar. 29, 1999	9	.367 " .026	.141 " .009	1	.428 " .041	.192 " .049
Apr. 12, 1999	9	.369 " .026	.152 " .009	2	.381 " .029	.160 " .035
Apr. 26, 1999	9	.381 " .026	.163 " .009	4	.347 " .019	.111 " .023
May 7, 1999	9	.473 " .026	.168 " .009	4	.350 " .019	.112 " .023

[1]IGGP = optical density reading in cows corrected for positive control per plate.
[2]IGGN = optical density reading in cows corrected for negative control per plate.
[3]IGGPC = optical density reading in calves corrected for positive control per plate.
[4]IGGNC = optical density reading in calves corrected for negative control per plate.

TABLE 9. Least Squares Means and SE for IgG Immune Response Specific to Fire Ant Venom Across Time in Gestating Ewes and Their Lambs

	Ewes			Lambs		
Date	n	IGGP[1]	IGGN[2]	n	IGGPL[3]	IGGNL[4]
Mar. 15, 1999	3	.206 " .011	.057 " .013	4	.177 " .006	.056 " .006
Mar. 22 1999	3	.204 " .011	.054 " .013	2	.172 " .009	.056 " .009
Mar. 29, 1999	9	.198 " .006	.053 " .007	11	.168 " .004	.052 " .004
Apr. 6, 1999	14	.222 " .005	.063 " .005	15	.158 " .003	.040 " .003
Apr. 12, 1999	15	.207 " .005	.045 " .005	18	.158 " .003	.040 " .003
May 3, 1999	12	.212 " .005	.046 " .006	19	.145 " .003	.026 " .003

[1]IGGP = optical density reading in ewes corrected for positive control per plate.
[2]IGGN = optical density reading in ewes corrected for negative control per plate.
[3]IGGPL = optical density reading in lambs corrected for positive control per plate.
[4]IGGNL = optical density reading in lambs corrected for negative control per plate.

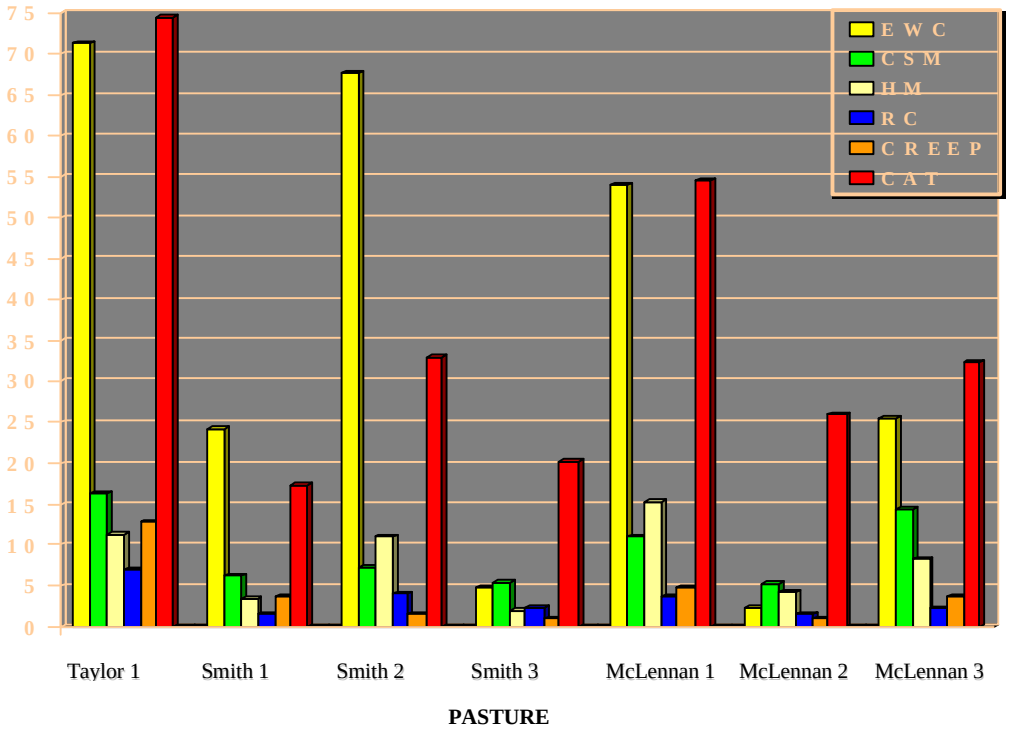

FIGURE 1. Mean Ant Count after 15 Minutes by Feed and Location

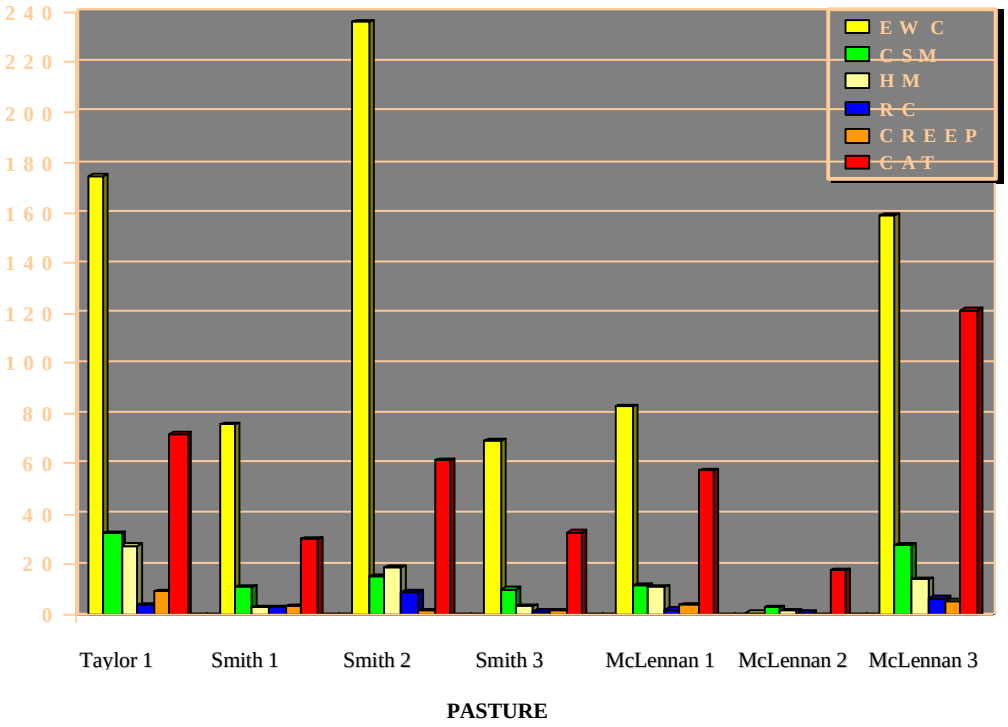

FIGURE 2. Mean Ant Count after 30 Minutes by Feed and Location