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STUDYING THE HANDLING OF HEAT STRESSED CATTLE USING THE ADDITIVE BI-LOGISTIC MODEL TO FIT BODY TEMPERATURE

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**STUDYING THE HANDLING OF HEAT STRESSED
CATTLE
USING THE ADDITIVE BI-LOGISTIC MODEL TO FIT
BODY TEMPERATURE**

By

Fan Yang

A Thesis

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**STUDYING THE HANDLING OF HEAT STRESSED
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Fan Yang, M.S.

University of Nebraska, 2011

Advisor: Anne M. Parkhurst

Daily activities consume the energy of heifers, subsequently causing an elevation of body temperature, depending on the ambient conditions. A better understanding of the dynamics of body temperature (T_b) would be helpful when deciding how to process and handle heifers. It would also lead to specific recommendations on moving heifers under different ambient conditions, especially during the summer. In this study, a bi-logistic mixed model is used to describe the dynamics of T_b during the moving event. Data were taken from heifers in pens located at different distances from the heifer work station on four separate summer days under hot conditions. This bi-logistic model has seven biological parameters: initial body temperature, heat challenge rate constant, upper asymptote body temperature, challenge

inflection point, baseline body temperature for recovery, recovery rate constant, and recovery inflection point. Pen and day were used as treatment factors in the model. Significant interactions between the factors were found for several parameters, indicating distance moved during the handling event influences the way an animal responds to a thermal challenge. The objectives of this study are to fit a bi-logistic mixed model for Tb with the above seven parameters, and to examine fixed and random effects. The main focus is to estimate and interpret the interactions between pens and days for the significant parameters to aid in management decisions involving when to work cattle.

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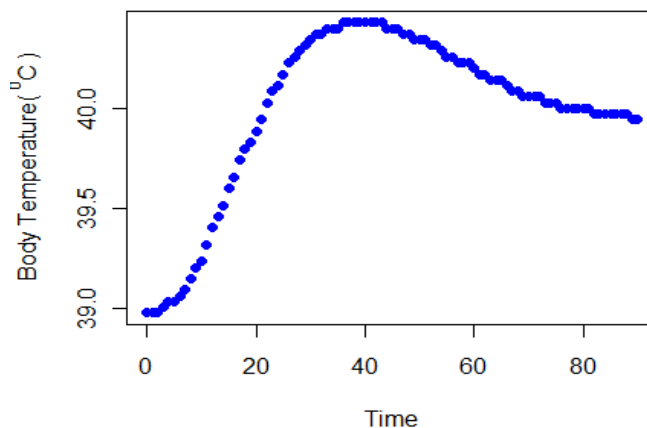
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1. INTRODUCTION

The cattle industry is one of the most important and profitable industries in the U.S. Profit from cattle industry is related to an animal's condition and environment. Heat stress can be a serious problem for cattle in a hot working environment. It is the major cause of lost profits in hostile "production-limiting" regions. Animals suffer heat stress when temperature exceeds some threshold. When heat stress happens, it will slow cattle growth and reduce cattle feeding and production, and will even result in death of susceptible cattle. Eventually, it will bring large economic losses. (Aitha et al. 2008) Therefore, understanding when an animal is experiencing heat stress can provide a basis for initiation of management practices to improve an animal's well being. (Davis et al. 2003)

Body temperature (T_b) is a good way to measure an animal's thermo-regulatory response to the environment. During heat stress, heifer's T_b increases from the initial T_b until it reaches some maximum as time goes by, then the T_b falls to some recovery T_b . (Figure 1) It found that heat stress in working cattle can be measured as the temperature differential, i.e., the difference between the upper asymptote and initial body temperature. Other parameters, such as challenge rate constant, recovery rate constant, challenge inflection point, recovery inflection point, recovery baseline body temperature, maximum body temperature, and time to reach maximum body temperature, are also associated with heat production. Parkhurst and Mader (2000) showed fitting parameters of a nonlinear bi-logistic model to T_b provides insight into the process of handling and moving animals during thermal challenge conditions of summer.

Figure 1 Heifer's body temperature ($^{\circ}\text{C}$) during heat stress measured over 90 minutes.



In this study, a nonlinear mixed bi-logistic model with seven parameters (initial T_b , upper asymptote T_b , recovery T_b , rates of challenge and recovery, inflation points for challenge and recovery) is used to describe heifer's body temperature during a moving event in a hot environment. Two parameters, maximum T_b and time for heifer to reach maximum T_b , which are important to estimate heat stress, can be derived from the bi-logistic model. A cross-over experimental design using pen (or distance) and day as factors is analyzed. The objectives of this study are: 1) to derive a 7-parameter bi-logistic model which can indicate the dynamics of T_b during thermal challenge and recovery, 2) to fit an appropriate mixed bi-logistic model with pen (or distance) and day treatment factors, 3) to examine the fixed and random effects in the mixed model and examine the pen-day interactions on 7 estimated parameters and 2 derived parameters, 4) to compare the nonlinear mixed model and the simplistic model, 5) to compare SAS and R results in the nonlinear mixed model.

2. MATERIALS and METHODS

2.1. Materials and Experimental Design

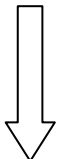
The data consisted of measurements taken from thirty-two 4-breed composite heifers ($\frac{1}{4}$ Pinzgauer, $\frac{1}{4}$ Red Poll, $\frac{1}{4}$ Hereford, and $\frac{1}{4}$ Angus; initially weighing 420 ± 43.9 kg). Heifers were randomly assigned to one of four pens on the basis of weight and health history (Brown-Brandl et al. 2009). Over the 10 week trial period, the experiment was conducted on two separate dates (6/26/2007, 8/1/2007) which had different average ambient temperatures during a moving event where the animals were processed in a squeeze chute (Table 1).

Table 1 Summary of weather data during each data collection period, starting at the time the animals left their pens and continuing through the end of the behavior data collection.

Date	Start time	End time	Dry-bulb Temperature	Dew-point Temperature
6/26/2007	9:58	13:58	26.8 °C	17.2 °C
8/1/2007	10:17	14:17	28.2 °C	23.0 °C

Pens were equally spaced approximately 160 – 200 m from a working facility. Pen 2 was located the furthest away, while pen 8 was the closest to the facility. During each working event, heifers were moved at about 11am from their pen to the working facility during a hot environmental challenging. Body temperature were recorded from 60 minutes prior to leaving the pen and then for the next 4 hours and on a one minute basis. In this study, body temperature data for 90 minutes after heifers were moved were analyzed, Table 2. A 5% significance level was chosen for all analyses in this study.

Table 2 32 heifers randomly assigned to 4 pens on 2 days.

Distance		6/26/2007	8/1/2007
		Cool	Hot
Pen 2	Furthest 200 m  Closest 160 m	Heifers 1-8	Heifers 1-8
Pen 4		Heifers 9-16	Heifers 9-16
Pen 6		Heifers 17-24	Heifers 17-24
Pen 8		Heifers 25-32	Heifers 25-32

2.2. Model

Parkhurst and Mader (2000) used a logistic relationship to describe the dynamics of Tb during thermal challenge or recovery. Parkhurst and Mader also suggested that a nonlinear mixed bi-logistic model could be used to describe Tb during the handling process. Kerek et al. (2003) built a segment bi-logistic model to estimate body temperature in feedlot cattle during heat stress. Li et al. (2009) compared experimental designs using a bi-logistic model without considering the initial body temperature to estimate heat stress when moving feedlot cattle. To provide a better prediction and interpretation, an improved bi-logistic model was derived and treatment effects need to be analyzed.

2.2.1 Background for Building an Additive Bi-Logistic Model

When an animal experiences a thermal challenge, the relative rate of heat accumulation is not constant but is proportional to the amount of heat remaining from the upper

asymptote Tb ; i.e. a logistic relationship, (Parkhurst and Mader 2000). Similarly, when an animal eliminates heat during thermal recovery, the relative recovery rate is proportional to the amount of heat above the baseline. Assuming that challenge and recovery are independent, an additive bi-logistic model is obtained by combining both logistic relationships. The two processes can be thought of as describing thermal challenge and thermal recovery.

2.2.1.1 Individual Logistic Functions

During the thermal challenge period, let $Y_{challenge}(t) = Tb(t) - \beta_1$ be the heat accumulation at time t , where Tb ($^{\circ}C$) is the body temperature at time t (min), which starts at 0 min; α is the upper asymptote Tb ($^{\circ}C$) indicating the highest Tb that heifer was expected to achieve in the absence of a recovery process; β_1 ($^{\circ}C$) is the lower asymptote Tb during thermal challenge, which is the lowest body temperature in the 60 min's observation period before heifer leaves the pen. Then,

$$\frac{\partial Y_{challenge}}{Y_{challenge} \partial t} = k_1 (\alpha - \beta_1 - Y_{challenge})$$

with $\frac{\partial Y_{challenge}}{Y_{challenge} \partial t} > 0$; $\alpha - \beta_1 > Y_{challenge}$; $k_1 > 0$.

Suppose $Tb(0) = Tb_{init}$, (i.e., Tb at $t=0$ is Tb_{init} , and $Tb_{init} \neq \beta_1$), then the initial condition is $Y_{challenge}(0) = Tb(0) - \beta_1 = Tb_{init} - \beta_1$.

Hence,

$$Y_{challenge}(t) = \frac{\alpha - \beta_1}{1 + e^{-k_1 t} \left(\frac{\alpha - Tb_{init}}{Tb_{init} - \beta_1} \right)}$$

$$\Leftrightarrow Tb(t) = \frac{\alpha - \beta_1}{1 + e^{-k_1 t} \left(\frac{\alpha - Tb_{init}}{Tb_{init} - \beta_1} \right)} + \beta_1$$

Let $\frac{\alpha - Tb_{init}}{Tb_{init} - \beta_1} = e^{k_1 \tau_1}$, then $\beta_1 = Tb_{init} - (\alpha - Tb_{init})e^{-k_1 \tau_1}$, where τ_1 is the inflection (or change)

point during the thermal challenge period.

Hence, when an animal experiences a thermal challenge (Figure 2 left), Tb can be written as

$$Tb(t) = \frac{(\alpha - Tb_{init})(1 - e^{-k_1 t})}{1 + e^{-k_1(t - \tau_1)}} + Tb_{init} \quad (1)$$

Similarly for thermal recovery:

Let $Y_{recovery}(t) = Tb(t) - \beta_2$ be the heat above the baseline at time t , where $Tb(t)$ is the body temperature at time t ; α is the upper asymptote Tb with the same meaning as in thermal challenge; β_2 ($^{\circ}\text{C}$) is the lower asymptotic Tb during thermal recovery, which is the lowest body temperature that heifer would achieve in the absence of the thermal challenge. Then,

$$\frac{\partial Y_{recovery}}{Y_{recovery} \partial t} = -k_2 (\alpha - \beta_2 - Y_{recovery})$$

with, $\frac{\partial Y_{recovery}}{Y_{recovery} \partial t} \leq 0$; $\alpha - \beta_2 \geq Y_{recovery}$; $k_2 > 0$

Suppose the effect of handling lasts 90min, then $Tb(90) = \delta$ when time is 90 (i.e. Tb at the ending point of the handling event is δ), then $Y_{recovery}(90) = Tb(90) - \beta_2 = \delta - \beta_2$.

Hence,

$$Y_{recovery}(t) = \frac{\alpha - \beta_2}{1 + e^{k_2(t-90)} \left(\frac{\alpha - \delta}{\delta - \beta_2} \right)}$$

$$\Leftrightarrow Tb(t) = \frac{\alpha - \beta_2}{1 + e^{k_2(t-90)} \left(\frac{\alpha - \delta}{\delta - \beta_2} \right)} + \beta_2$$

Let $e^{-k_2 90} \frac{\alpha - \delta}{\delta - \beta_2} = e^{-k_2 \tau_2}$, then $\beta_2 = \delta - (\alpha - \delta)e^{-k_2(90 - \tau_2)}$, where τ_2 is the inflection (or

change) point for recovery.

Hence, when an animal experiences thermal recovery (Figure 2 middle) is

$$Tb = \frac{(\alpha - \delta)(1 - e^{-k_2(90-t)})}{1 + e^{k_2(t-\tau_2)}} + \delta \quad (2)$$

2.2.1.2 Additive Bi-Logistic Function

Viewing the process as a whole, the differential equation can be considered to be the sum of the rate of change in each process. Thus, the overall rate of change is actually the rate of challenge plus the rate of recovery. It is a combination of two logistic processes.

Therefore, it is named an additive bi-logistic function.

$$\frac{\partial Tb}{\partial t} = \frac{\partial Tb}{\partial t} \Big|_{challenge} + \frac{\partial Tb}{\partial t} \Big|_{recovery} = \frac{\partial(Y_{challenge} + \beta_1)}{\partial t} + \frac{\partial(Y_{recovery} + \beta_2)}{\partial t} = \frac{\partial Y_{challenge}}{\partial t} + \frac{\partial Y_{recovery}}{\partial t}$$

That is,

$$\frac{\partial Tb}{\partial t} = k_1 Y_{challenge} (\alpha - \beta_1 - Y_{challenge}) - k_2 Y_{recovery} (\alpha - \beta_2 - Y_{recovery})$$

with initial condition $Tb(0) = Tb_{init}$ and ending condition $Tb(90) = \delta$. Then,

$$Tb(t) = \alpha - (\alpha - Tb_{init}) \left(\frac{e^{-k_1 \tau_1} + 1}{e^{k_1(t-\tau_1)} + 1} \right) - (\alpha - \delta) \left(\frac{e^{k_2(\tau_2-90)} + 1}{e^{-k_2(t-\tau_2)} + 1} \right) \quad (3)$$

where $\alpha, \delta, Tb_{init}, k_1, k_2, \tau_1$, and τ_2 are all positive. (Figure 2 right)

α is the upper asymptotic body temperature (not the maximum Tb),

Tb_{init} is animal's initial body temperature

δ is animal's baseline body temperature for recovery.

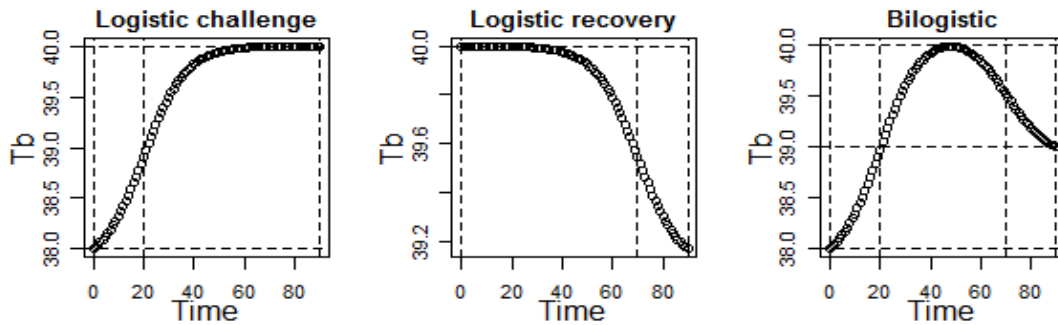
k_1 is the rate constant for challenge period.

k_2 is the rate constant for recovery period.

τ_1 is the inflection points for challenge period.

τ_2 is the inflection points for recovery period.

Figure 2 Plots of simulated logistic models and simulated additive Bi-logistic model, for Tb over 90 min using $\alpha=40.015$ (such that $Tb_{max}=40$), $Tb_{init}=38$, $\delta=39$, $k_1=0.12$, $k_2=0.12$, $\tau_1=20$, $\tau_2=70$.



2.2.2 Mixed Additive Bi-logistic Model with Treatment Effects

The additive bi-logistic function provides a way to describe Tb during the handling event.

A nonlinear mixed model with fixed effects and random heifer effects needs to be considered. For the data in this study, the 4 by 2 factorial treatment effects were executed in split-plot design. The desired random structure is the one that heifer within pen as the whole plot error and day by whole plot error as the subplot error. Given the nonlinear nature of the bi-logistic model, there are many complications inherent in fitting the

desired random structure. There are also philosophical considerations. While animals were housed in the same pen and moved at the same time, their Tb was measured individually and they did not move in lock-step. In an effort to simplify the analysis, several random structures were explored. The potential random structure is heifer within pen within day. This model failed to converge even after an extensive exploration of starting values and alternative optimization routines. Another structure involving heifer and heifer within day crossed with pen also failed to converge.

One way of addressing the randomization restriction on heifers is to question the maturation process. Can the group of heifers on the cooler day in June be considered to be the same group on a hotter day in July or is there evidence the variation is nested? Assumed they are not, two random structures, hence, were considered.

2.2.2.1 Random Heifer Effects

For one model the grouping structure factors are said to be crossed (individual cluster) if every level of one factor occurs with every level of another factor, and vice versa. The structure is considered to be nested within another factor (Hierarchical cluster) if every level of this factor occurs with only one level of the other factor. (Zhou et al. 2006)

2.2.2.1.i. Individual Clusters or Crossed Random Structure

In this structure, each factor combination is considered a separate group. Data were grouped by a factor “heiferDay” which was a combination of heifer i.d. and day number. Thus, the random effect in this structure was clustered by heiferDay.

The statistical model for individual clusters (crossed) structure is

$$Tb_i = \alpha - (\alpha - Tb_{init}) \left(\frac{e^{-k_1 \tau_1} + 1}{e^{k_1(t-\tau_1)} + 1} \right) - (\alpha - \delta) \left(\frac{e^{k_2(\tau_2-90)} + 1}{e^{-k_2(t-\tau_2)} + 1} \right) + \varepsilon_i \quad (4)$$

where

$$\left[\begin{array}{l} \alpha = \mu_\alpha + b_{\alpha:heiferDay} \\ \delta = \mu_\delta + b_{\delta:heiferDay} \\ k_1 = \mu_{k_1} + b_{k_1:heiferDay} \\ k_2 = \mu_{k_2} + b_{k_2:heiferDay} \\ \tau_1 = \mu_{\tau_1} + b_{\tau_1:heiferDay} \\ \tau_2 = \mu_{\tau_2} + b_{\tau_2:heiferDay} \\ Tb_{init} = \mu_{init} + b_{init:heiferDay} \end{array} \right], \text{ and}$$

$$\Psi_{heiferDay} = \left[\begin{array}{ccccccc} \psi_{\alpha:heiferDay} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \psi_{\delta:heiferDay} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \psi_{k_1:heiferDay} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \psi_{k_2:heiferDay} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \psi_{\tau_1:heiferDay} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \psi_{\tau_2:heiferDay} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \psi_{init:heiferDay} \end{array} \right]$$

$$\boldsymbol{\mu} = \left(\begin{array}{c} \mu_\alpha \\ \mu_\delta \\ \mu_{k_1} \\ \mu_{k_2} \\ \mu_{\tau_1} \\ \mu_{\tau_2} \\ \mu_{init} \end{array} \right)$$

are fixed effects which represent the population means.

$$\mathbf{b}_{heiferDay} = \begin{pmatrix} b_{\alpha:heiferDay} \\ b_{\delta:heiferDay} \\ b_{k_1:heiferDay} \\ b_{k_2:heiferDay} \\ b_{\tau_1:heiferDay} \\ b_{\tau_2:heiferDay} \\ b_{init:heiferDay} \end{pmatrix}$$

are the random effects at heiferDay level. They present the deviations from the population mean associated with different heiferDay handling events.

$\mathbf{b}_{heiferDay} \sim N(0, \Psi_{heiferDay})$ and $\boldsymbol{\varepsilon} \sim N(0, \boldsymbol{\sigma}^2 \mathbf{I})$ are independent from each other.

2.2.2.1.ii Hierarchical Clusters or Nested random structure

In a hierarchical clusters structure, the sub-samples are nested within the level-one sampling units. In this structure, data were grouped by “heifer/day”. Thus the random effect has 2 levels: heifer and day within heifer. The nested random structure with grouping factor “heifer/day” helps to access the interaction between heifer and day.

The additive bi-logistic model with both fixed and random effects for the nested structure is

$$Tb_i = \alpha - (\alpha - Tb_{init}) \left(\frac{e^{-k_1 \tau_1} + 1}{e^{k_1(t-\tau_1)} + 1} \right) - (\alpha - \delta) \left(\frac{e^{k_2(\tau_2-90)} + 1}{e^{-k_2(t-\tau_2)} + 1} \right) + \boldsymbol{\varepsilon}_i, \quad (5)$$

$$\text{where } \begin{bmatrix} \alpha & = & \mu_{\alpha} + b_{\alpha:\text{heifer}} + b_{\alpha:\text{day}(\text{heifer})} \\ \delta & = & \mu_{\delta} + b_{\delta:\text{heifer}} + b_{\delta:\text{day}(\text{heifer})} \\ k_1 & = & \mu_{k_1} + b_{k_1:\text{heifer}} + b_{k_1:\text{day}(\text{heifer})} \\ k_2 & = & \mu_{k_2} + b_{k_2:\text{heifer}} + b_{k_2:\text{day}(\text{heifer})} \\ \tau_1 & = & \mu_{\tau_1} + b_{\tau_1:\text{heifer}} + b_{\tau_1:\text{day}(\text{heifer})} \\ \tau_2 & = & \mu_{\tau_2} + b_{\tau_2:\text{heifer}} + b_{\tau_2:\text{day}(\text{heifer})} \\ Tb_{\text{init}} & = & \mu_{\text{init}} + b_{\text{init}:\text{heifer}} + b_{\text{init}:\text{day}(\text{heifer})} \end{bmatrix},$$

$$\Psi_{\text{heifer}} = \begin{bmatrix} \psi_{\alpha:\text{heifer}} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \psi_{\delta:\text{heifer}} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \psi_{k_1:\text{heifer}} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \psi_{k_2:\text{heifer}} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \psi_{\tau_1:\text{heifer}} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \psi_{\tau_2:\text{heifer}} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \psi_{\text{init}:\text{heifer}} \end{bmatrix} \text{ and}$$

$$\Psi_{\text{day}(\text{heifer})} = \begin{bmatrix} \psi_{\alpha:\text{day}(\text{heifer})} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & \psi_{\delta:\text{day}(\text{heifer})} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \psi_{k_1:\text{day}(\text{heifer})} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \psi_{k_2:\text{day}(\text{heifer})} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \psi_{\tau_1:\text{day}(\text{heifer})} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \psi_{\tau_2:\text{day}(\text{heifer})} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & \psi_{\text{init}:\text{day}(\text{heifer})} \end{bmatrix}$$

$$\boldsymbol{\mu} = \begin{pmatrix} \mu_{\alpha} \\ \mu_{\delta} \\ \mu_{k_1} \\ \mu_{k_2} \\ \mu_{\tau_1} \\ \mu_{\tau_2} \\ \mu_{\text{init}} \end{pmatrix}$$

are fixed effects of all seven parameters in the mixed model.

$$\mathbf{b}_{heifer} = \begin{pmatrix} b_{\alpha:heifer} \\ b_{\delta:heifer} \\ b_{k_1:heifer} \\ b_{k_2:heifer} \\ b_{\tau_1:heifer} \\ b_{\tau_2:heifer} \\ b_{init:heifer} \end{pmatrix}$$

are the random effects of heifer. They represent the deviations from the population mean associated with heifer.

$$\mathbf{b}_{day(heifer)} = \begin{pmatrix} b_{\alpha:day(heifer)} \\ b_{\delta:day(heifer)} \\ b_{k_1:day(heifer)} \\ b_{k_2:day(heifer)} \\ b_{\tau_1:day(heifer)} \\ b_{\tau_2:day(heifer)} \\ b_{init:day(heifer)} \end{pmatrix}$$

are the random effect of day within heifer level. They represent the deviations from the population mean associated with each day for a heifer.

$\mathbf{b}_{heifer} \sim N(0, \Psi_{heifer})$, $\mathbf{b}_{day(heifer)} \sim N(0, \Psi_{day(heifer)})$ and $\boldsymbol{\varepsilon} \sim N(0, \boldsymbol{\sigma}^2 \mathbf{I})$ are independent from each other.

2.2.2.2 Fixed Treatment Effects

In this study, there were 2 treatment factors, day (2 levels) and pen (4 levels), which designate a 4x2 factorial treatment design. To determine if pen (distance) and Day have an effect on Tb, fixed treatment effects need to be included in the model.

The statistic models for both random effect structures with treatment effect were given in (6) and (7).

$$Tb(t) = \alpha - (\alpha - Tb_{init}) \left(\frac{e^{-k_1 \tau_1} + 1}{e^{k_1(t-\tau_1)} + 1} \right) - (\alpha - \delta) \left(\frac{e^{k_2(\tau_2-90)} + 1}{e^{-k_2(t-\tau_2)} + 1} \right) + \boldsymbol{\varepsilon}$$

In an individual cluster (crossed) structure, if i represents date and j represents the pen number, then

$$\begin{aligned}
 \alpha &= \bar{\alpha} + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\alpha,ij} + b_{\alpha:heiferDay} \\
 \delta &= \bar{\delta} + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\delta,ij} + b_{\delta:heiferDay} \\
 k_1 &= \bar{k}_1 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{k_1,ij} + b_{k_1:heiferDay} \\
 k_2 &= \bar{k}_2 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{k_2,ij} + b_{k_2:heiferDay} \\
 \tau_1 &= \bar{\tau}_1 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\tau_1,ij} + b_{\tau_1:heiferDay} \\
 \tau_2 &= \bar{\tau}_2 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\tau_2,ij} + b_{\tau_2:heiferDay} \\
 Tb_{init} &= \overline{Tb}_{init} + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{init,ij} + b_{init:heiferDay}
 \end{aligned} \tag{6}$$

$$\Psi_{heiferDay} = \begin{bmatrix}
 \psi_{\alpha:heiferDay} & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & \psi_{\delta:heiferDay} & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & \psi_{k_1:heiferDay} & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & \psi_{k_2:heiferDay} & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & \psi_{\tau_1:heiferDay} & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & \psi_{\tau_2:heiferDay} & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & \psi_{init:heiferDay}
 \end{bmatrix}$$

$$\begin{bmatrix}
 \bar{\alpha} \\
 \bar{\delta} \\
 \bar{k}_1 \\
 \bar{k}_2 \\
 \bar{\tau}_1 \\
 \bar{\tau}_2 \\
 \overline{Tb}_{init}
 \end{bmatrix}$$

are the overall means for all parameters.

$$C_{ij} = \begin{cases} 1, & \text{if } j^{\text{th}} \text{ pen on } i^{\text{th}} \text{ day presents} \\ 0, & \text{otherwise} \end{cases}$$

$$\begin{bmatrix} \beta_{\alpha,ij} \\ \beta_{\delta,ij} \\ \beta_{k_1,ij} \\ \beta_{k_2,ij} \\ \beta_{\tau_1,ij} \\ \beta_{\tau_2,ij} \\ \beta_{init,ij} \end{bmatrix}$$

are the treatment effects on the i^{th} day and in j^{th} pen for all parameters.

$$\mathbf{b}_{heiferDay} = \begin{pmatrix} b_{\alpha:heiferDay} \\ b_{\delta:heiferDay} \\ b_{k_1:heiferDay} \\ b_{k_2:heiferDay} \\ b_{\tau_1:heiferDay} \\ b_{\tau_2:heiferDay} \\ b_{init:heiferDay} \end{pmatrix}$$

are the random effects at heiferDay level. They present the deviations from the population mean associated with different heiferDay handling events.

$\mathbf{b}_{heiferDay} \sim N(0, \Psi_{heiferDay})$ and $\boldsymbol{\varepsilon} \sim N(0, \boldsymbol{\sigma}^2 \mathbf{I})$ are independent with each other.

In a Hierarchical clusters (nested) structure,

$$\begin{aligned}
 \alpha &= \bar{\alpha} + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\alpha,ij} + b_{\alpha:heifer} + b_{\alpha:day(heifer)} \\
 \delta &= \bar{\delta} + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\delta,ij} + b_{\delta:heifer} + b_{\delta:day(heifer)} \\
 k_1 &= \bar{k}_1 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{k_1,ij} + b_{k_1:heifer} + b_{k_1:day(heifer)} \\
 k_2 &= \bar{k}_2 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{k_2,ij} + b_{k_2:heifer} + b_{k_2:day(heifer)} \\
 \tau_1 &= \bar{\tau}_1 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\tau_1,ij} + b_{\tau_1:heifer} + b_{\tau_1:day(heifer)} \\
 \tau_2 &= \bar{\tau}_2 + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{\tau_2,ij} + b_{\tau_2:heifer} + b_{\tau_2:day(heifer)} \\
 Tb_{init} &= \bar{Tb}_{init} + \sum_{i=1}^2 \sum_{j=1}^4 C_{ij} \beta_{init,ij} + b_{init:heifer} + b_{init:day(heifer)}
 \end{aligned} \tag{7}$$

where

$$\Psi_{heifer} = \begin{bmatrix}
 \psi_{\alpha:heifer} & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & \psi_{\delta:heifer} & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & \psi_{k_1:heifer} & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & \psi_{k_2:heifer} & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & \psi_{\tau_1:heifer} & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & \psi_{\tau_2:heifer} & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & \psi_{init:heifer}
 \end{bmatrix}$$

and

$$\Psi_{day(heifer)} = \begin{bmatrix}
 \psi_{\alpha:day(heifer)} & 0 & 0 & 0 & 0 & 0 & 0 \\
 0 & \psi_{\delta:day(heifer)} & 0 & 0 & 0 & 0 & 0 \\
 0 & 0 & \psi_{k_1:day(heifer)} & 0 & 0 & 0 & 0 \\
 0 & 0 & 0 & \psi_{k_2:day(heifer)} & 0 & 0 & 0 \\
 0 & 0 & 0 & 0 & \psi_{\tau_1:day(heifer)} & 0 & 0 \\
 0 & 0 & 0 & 0 & 0 & \psi_{\tau_2:day(heifer)} & 0 \\
 0 & 0 & 0 & 0 & 0 & 0 & \psi_{init:day(heifer)}
 \end{bmatrix}$$

$$\begin{bmatrix} \bar{\alpha} \\ \bar{\delta} \\ \bar{k}_1 \\ \bar{k}_2 \\ \bar{\tau}_1 \\ \bar{\tau}_2 \\ \bar{Tb}_{init} \end{bmatrix}$$

are the overall means for all parameters.

$$C_{ij} = \begin{cases} 1, & \text{if } j^{\text{th}} \text{ pen on } i^{\text{th}} \text{ day presents} \\ 0, & \text{otherwise} \end{cases}$$

$$\begin{bmatrix} \beta_{\alpha,ij} \\ \beta_{\delta,ij} \\ \beta_{k_1,ij} \\ \beta_{k_2,ij} \\ \beta_{\tau_1,ij} \\ \beta_{\tau_2,ij} \\ \beta_{init,ij} \end{bmatrix}$$

are the treatment effects on the i^{th} day and in j^{th} pen for all parameters.

$$\mathbf{b}_{heifer} = \begin{pmatrix} b_{\alpha:heifer} \\ b_{\delta:heifer} \\ b_{k_1:heifer} \\ b_{k_2:heifer} \\ b_{\tau_1:heifer} \\ b_{\tau_2:heifer} \\ b_{init:heifer} \end{pmatrix}$$

are the random effects of heifer. They represent the deviations from the population mean associated with heifer.

$\mathbf{b}_{day(heifer)} = \begin{pmatrix} b_{\alpha:day(heifer)} \\ b_{\delta:day(heifer)} \\ b_{k_1:day(heifer)} \\ b_{k_2:day(heifer)} \\ b_{\tau_1:day(heifer)} \\ b_{\tau_2:day(heifer)} \\ b_{init:day(heifer)} \end{pmatrix}$

are the random effect of day within heifer level. They represent the deviations from the population mean associated with each day for a heifer.

$\mathbf{b}_{heifer} \sim N(0, \Psi_{heifer})$, $\mathbf{b}_{day(heifer)} \sim N(0, \Psi_{day(heifer)})$ and $\boldsymbol{\varepsilon} \sim N(0, \boldsymbol{\sigma}^2 \mathbf{I})$ are independent with each other.

2.2.2.3 Comparison of Mixed Bi-Logistic Models

After fixed treatment effects and random heifer effects were specified, the four models were compared using log-likelihood, Information Criteria (AIC and BIC), and fitted plots. The model with higher log-likelihood, smaller AIC and BIC, and no unusual residuals would be preferred. The likelihood ration test (LRT) was also constructed between two models to test if one was significantly better than the other.

The mixed bi-logistic model had seven parameters. Due to the difficulty in obtaining convergence for the optimization algorithm used in NLME (Pinheiro and Bates, 2000), a diagonal random structure was assumed for the random-effects covariance matrices. To identify which parameters, if any, require random effects, the mixed models in both cases were fit for several sets of diagonal random effects structures and checked for model equivalency. Each time, one random effect was removed; the reduced model was compared to the full model with all

diagonal random effects using the LRT. If the LRT is not significant, then the removal of random effect was justified.

2.3 Maximum Tb and Time to Reach Maximum Tb

Maximum body temperature and the time to reach the maximum Tb are two important parameters when making decisions during a moving event in a hot environment. Knowledge of the size and time it would take a heifer to reach its maximum body temperature would be helpful for a manager to develop a viable strategy before moving cattle so as to avoid economic loss.

The maximum Tb during the handling process occurs at the equilibrium between the heat challenge and recovery when the rate of change in Tb is zero. Using the additive bi-logistic function (3), $\frac{\partial Tb}{\partial t}$, is expressed as following:

$$\frac{\partial Tb}{\partial t} = (\alpha - Tb_{init}) \left(\frac{(e^{-k_1 \tau_1} + 1)(k_1 e^{k_1(t-\tau_1)})}{[e^{k_1(t-\tau_1)} + 1]^2} \right) - (\alpha - \delta) \left(\frac{(e^{k_2(\tau_2-90)} + 1)(k_2 e^{-k_2(t-\tau_2)})}{[e^{-k_2(t-\tau_2)} + 1]^2} \right) = 0 \quad (8)$$

Eq(8) can be solved for t using R function **uniroot**. (Brent, R. 1973) The solution is the estimated time to reach the maxTb, denoted by $t_{\max Tb}$. The corresponding body temperature at $t_{\max Tb}$ is the maximum Tb, denoted by maxTb. It is noticeable that standard errors for both maxTb and $t_{\max Tb}$ are not estimated in R due to the limitation of delta method.

2.4 Comparisons of R and SAS results

The procedure of building mixed bi-logistic models, models comparisons, examination of fixed and random effects, and parameters estimation were done in both R 2.11.1 and SAS 9.2. The major R functions used are NLS, NLIST, and NLME. (Ponheiro and Bates, 2000). The major SAS procedures used are PROC NLIN and PROC NLMIXED. (SAS Institute Inc. 2010). At the end of the analysis, results from both R and SAS are compared.

3. RESULTS and DISCUSSION

3.1 Mixed Additive Bi-Logistic Model

A mixed model with diagonal random-effects structure was used to examine heifer effects associated with each treatment factor. To find the best mixed model, comparisons were made between two groupings of random heifer effects, individual (crossed) and hierarchical (nested) clusters, both with and without treatment effects. The goodness-of-fit statistics for comparing the four models are summarized in Table 3.

For the models without treatment effects, the individual cluster (crossed) random-effects structure (Model 1) had smaller AIC, BIC, but larger log-likelihood compared to the (nested) clusters structure (Model 3) and the p-value for LRT was less than 5%. (not shown). Hence, a mixed additive bi-logistic model with crossed random effect structure was better than a nested random effect structure when treatment was not included.

For each random-effects structure comparisons were made between models with and without treatments effect. After treatment effects were included in the mixed model, AIC and BIC were smaller and log-likelihood statistics were larger when compared to models without treatment effect. For the both random-effects structures, the p-value for LRT was less than 5%. (Model 1 vs 3 and Model 2 vs 4, not shown). Hence, the treatment effect is needed in the model regardless of the random-effects structure.

Comparing model 4 (with treatment effects and nested random structure) with the other three models showed model 4 had the smallest AIC and BIC, largest log-likelihood and a better fit on the prediction plot, Fig 2. The p-values for three LRTs (Model No. 1 vs 4, 2 vs 4, and 3 vs 4) were all less than 5% which indicate the nested model with treatment effect was significantly better than the other three models. (Table 3) .

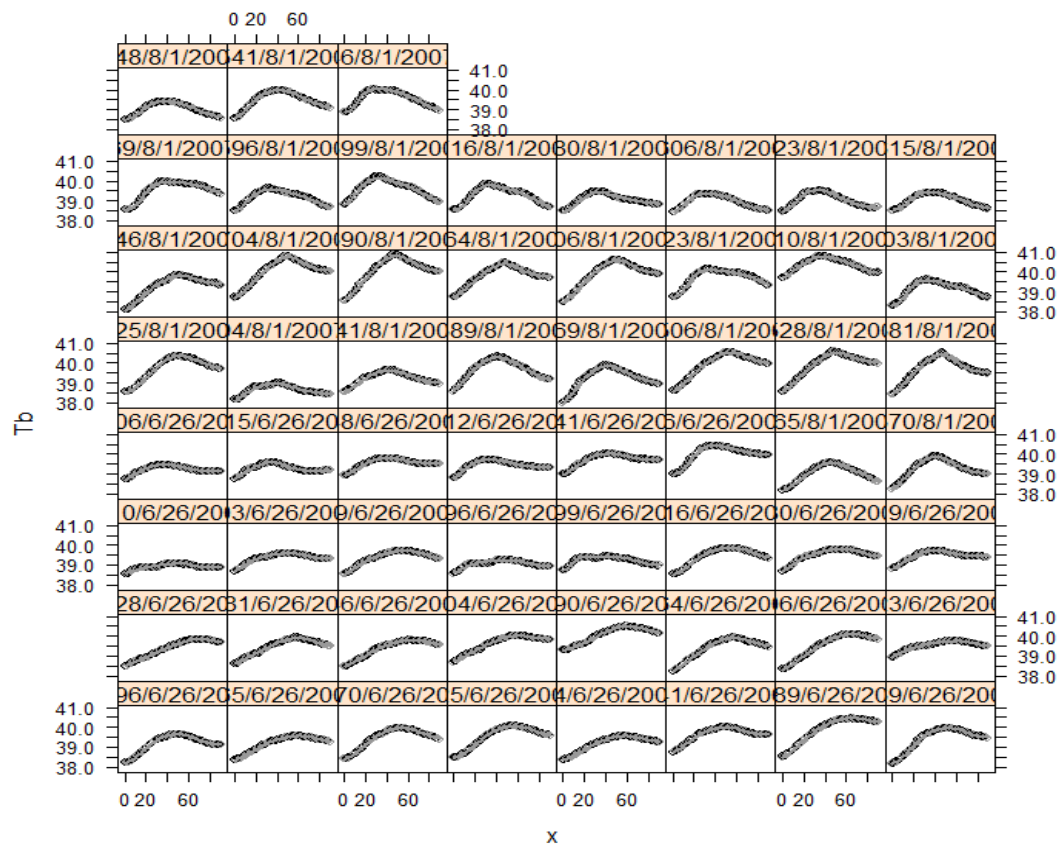
Table 3 Statistics from mixed models with crossed and nested diagonal random-effects structures with and without treatment effects.

Model		AIC	BIC	Log-likelihood	Test	Likeli. Ratio	P-value
No.	Structures						
1	Without trt effect & Crossed	-21095	-20996	10562	1 vs 4	395.67	<.0001
2	Without trt effect & Nested	-20689	-20544	10366	2 vs 4	787.73	<.0001
3	With trt effect & Crossed	-21322	-20900	10725	3 vs 4	70.912	<.0001
4	With trt effect & Nested	-21379	-20911	10760	--	--	--

Figure 3 showed that the predicted data provided a good fit to the observed data at each day within heifer level, though some were underestimated. The residual analysis (not shown) supported the assumptions that residuals from model 4 at each day within heifer level were

normal and randomly distributed about the zero line without any outliers. Therefore, the preferable model is the mixed bi-logistic model with nested cluster random effects.

Figure 3 Multiple plots for observed (black open circle) and predicted (grey solid line) Tb from mixed additive bi-logistic model with treatments and diagonal nested random-effect structure. Missing values were excluded.



3.2 Examination of Random Effects

The full diagonal random-effects structure specified in (7) was summarized in Table 4.

Estimated standard deviations for seven parameters at both heifer and day within heifer

levels are necessary (p-values from LRT test were significant (< 0.05) which showed that the effects cannot be removed from the random structure). The residual for model 4 was 0.02610 which was significantly smaller than the residual for model 3 (0.02725, not shown). The nested structure with the random-effects among heifer and day within heifer could account for the variation which is due to heifer conditions and environmental factors. The interactions between heifer and day within heifer existed in all parameters.

Table 4 Standard deviations of Random-effects and Residual for Nonlinear Mixed Models with Nested Random Structure

Parameters Levels	Standard Deviations							Residual
	A	δ	Tb_{init}	k_1	k_2	τ_1	τ_2	
Heifer	0.0044	0.0041	0.0025	0.1033	0.0001	0.1512	0.0680	0.02610
Day in heifer	0.0089	0.0064	0.0054	0.3412	0.2399	0.8450	0.3022	

3.3 Examination of Treatment Effects

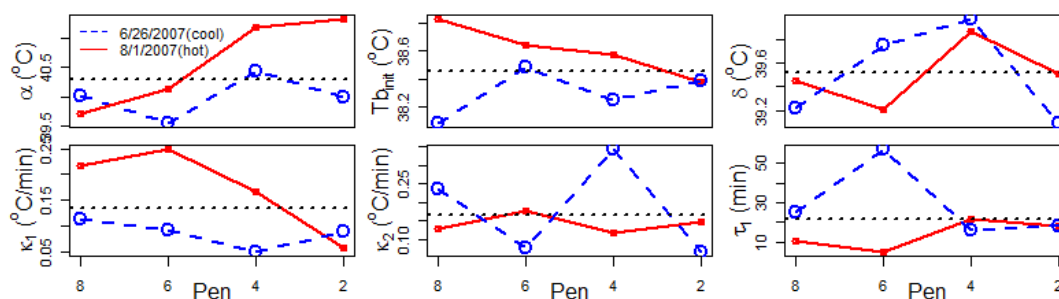
The mixed model with hierarchical random-effects was used to determine the significance of the treatment effects associated with each parameter. The results were summarized in Table 5. The p-values for interactions are all significant ($< 5\%$) except the recovery inflection point, τ_2 , which is essentially the change point - time at which the rate changes from decreasing to increasing. It indicates that there is no significant pen and day interaction for τ_2 . In addition, none of the Pen or Day effects are significant. The estimate of the fixed effect recovery change point (the inflection point of recovery) is 72.35 min.

Table 5 ANOVA for testing treatment effects for each parameter (denominator d.f.=5255).

Factor		Num DF	F-value	P-value
α	Intercept	1	4741237	<.0001
	Pen	3	10389	<.0001
	Day	1	56555	<.0001
	Pen X Day	3	151213	<.0001
δ	Intercept	1	10518321	<.0001
	Pen	3	1455	<.0001
	Day	1	3439	<.0001
	Pen X Day	3	3359	<.0001
Tb_{init}	Intercept	1	18656897	<.0001
	Pen	3	12	<.0001
	Day	1	40	<.0001
	Pen X Day	3	3	0.0151
k_1	Intercept	1	1777	<.0001
	Pen	3	9	<.0001
	Day	1	7	0.0065
	Pen X Day	3	6	0.0005
k_2	Intercept	1	3293	<.0001
	Pen	3	17	<.0001
	Day	1	33	<.0001
	Pen X Day	3	14	<.0001
τ_1	Intercept	1	563	<.0001
	Pen	3	6	0.0008
	Day	1	9	0.0036
	Pen X Day	3	3	0.0476
τ_2	Intercept	1	9725	<.0001
	Pen	3	2	0.0960
	Day	1	0	0.6358
	Pen X Day	3	2	0.0644

For all other parameters, the interactions between days and pens were examined. To help understand the significant day and pen interactions on the six parameters, α , δ , Tb_{init} , k_1 , k_2 , and τ_1 , plots for the treatment means were examined, Fig 3. For each parameter, the day means were given by pen. The pen with the shortest distance was given first. If there was no interaction, the day line segments in the plot would be parallel. It is clear that there was an interaction between pens and days for each parameter in Figure 4.

Figure 4 Interaction plots of parameters for cool day (blue dashed) and hot day (red solid). Pen closest to work station is given first. The overall mean (dotted) for each parameter is given.



The upper asymptotic body temperature, α , indicates the highest body temperature an animal is expected to achieve in the absence of a recovery process. The upper asymptote was higher for the hot day than it was for the cool day except for the pen 8 which was closest to the work station. An increasing trend was shown for the hot day as the distance of the pen from the work station increased. Overall, heifers moved farthest in a hot environment had a higher Tb asymptote.

The initial body temperature, Tb_{init} , was higher for the hot day than it was for the cool day except for the most distant pen 2. For that pen, the two days had essentially the same initial body temperature. This suggests heifer exposed in higher ambient temperature would have a higher initial body temperature during heat stress.

Heifers in pens 6 and 4 had higher baseline Tb for recovery, δ , on the cooler day. The baseline Tb for recovery in pen 4 was significantly higher than others (p-value=0.0256). It was above the overall mean on both days. This suggests the presence of other factors

related to pen that interfere with the heifers' recovery and is a topic for further investigation.

The interaction plot of the initial challenge rate constant, k_1 , showed a decreasing trend for the hot day. A higher initial challenge rate constant indicates a more rapid increase in Tb. On a hot day, heifers moved a short distance had a higher challenge rate constant, which implies Tb increased more rapidly than for heifers moved a longer distance. This result suggests that when heifers were challenged by heat stress, they took longer to adjust to handling the farther they were moved.

The recovery rate constant, k_2 , did not change noticeably for the hot day; for the cool day, the rate constant was significantly different from the rest in pen 4. The recovery rate constant in pen 4 was significantly higher than the overall mean (p-value=0.0001). Pen 4 was previously a matter of concern when considering the recovery baseline Tb. Once again this suggests the presence of other factors related to Pen 4 that hamper the heifers' recovery and is a topic for further investigation.

There was a significant interaction for the challenge inflection point, τ_1 , which is essentially the change point - time at which the rate changes from increasing to decreasing. This parameter is usually associated with the challenge rate constant. On the cool day, τ_1 was significantly higher for the closest pens (8 and 6) while their rate constants were lower. This implies that heifers may need more time to adjust to the stress of handling when they were moved a short distance during a cool working environment.

The opposite is true for the hot day. The heifer's inflection point is lower while the initial rate constant is higher suggesting heifers need to respond more rapidly to the moving event.

3.4 Examination of Maximum Tb and Time to Reach Maximum Tb

Multiple plots for predicted Tb with estimated maxTb and $t_{\max\text{Tb}}$ were shown in Fig 4. Estimates of $t_{\max\text{Tb}}$ were missing for two heifers (in pen 1 on 6/26/2007 and in pen 8 on 6/26/2007), because it failed to converge when solving eqn (8) due to the convergence criteria. The treatment means for the day by pen interaction plots are shown in Figure 6. Animals in the most distant pens, 2, 4, and 6 needed significantly more time to reach their maximum body temperature, $t_{\max\text{Tb}}$, on cool days than on hot days (p-values = 0.0001823, 0.0001132, 0.001574, respectively). Only in pen 8 (closest), there was no significant difference between two days (p-value = 0.06857). This implies that heifers moved a short distance will reach their maximum Tb quickly no matter whether the weather is hot or cool. It is noticeable that $t_{\max\text{Tb}}$ is larger for heifers in pen 4 on both days. This suggests the presence of other factors, such as heifer's condition may also effect response time.

Other factors may also be responsible for the significant difference in Maximum Tb, maxTb, between cool and hot days in pen 6 (p-value=0.006044). There were no significant difference in maxTb for heifers in pen 2, 4 and 8 (p-values= 0.5617, 0.1785, 0.219, respectively). Therefore, maximum Tb is higher when heifer is in a hot environment. Moving distance does not significantly affect on heifers maximum Tb.

These results suggest that heifers tend to fluctuate around mean maxTb regardless of day or distance and it is more important to consider the time to reach maxTb when formulating management strategies.

Figure 5 Multiple plots for raw Tb and predicted Tb with estimated maximum body temperature, maxTb, and time to reach maximum body temperature, t_{maxTb} . Red line - Raw data; Black dashed line-Fitted data.

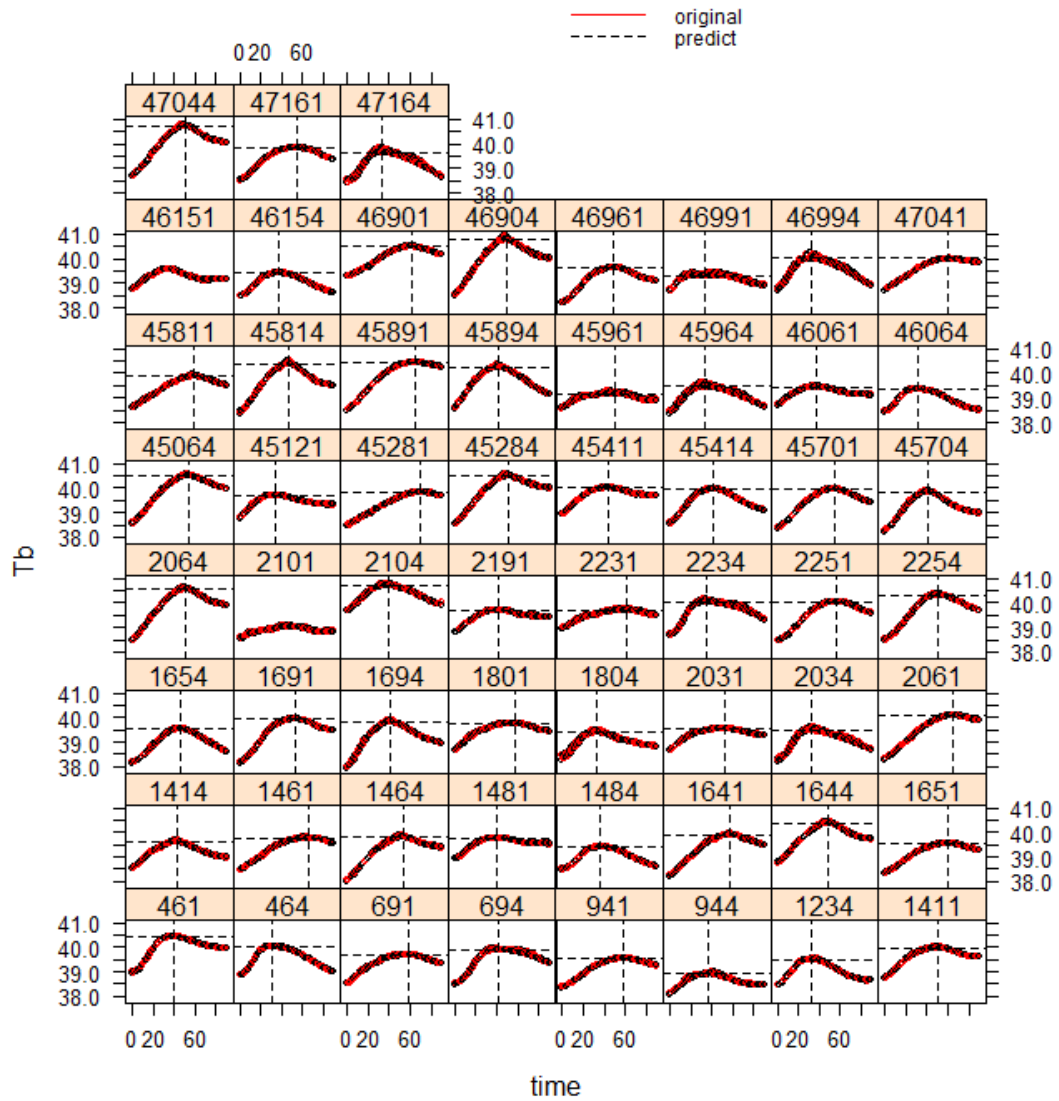
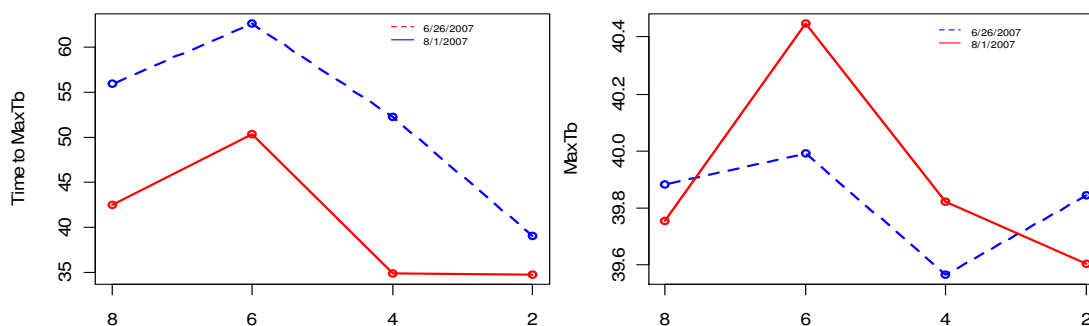


Figure 6 Interaction plots for 2 estimated parameters, time to reach maximum Tb, $t_{\max Tb}$, (left) and maximum Tb, $\max Tb$, (right), on cool day (blue dashed) and hot day (red solid). Pen closest to work station is given first.



3.5 Comparisons of Simplistic approach and Nonlinear approach

Statistics from simplistic approach were concluded in Table 6. (Brown-Brandl et al. 2009) There are significant interactions between pen (distance) and day on initial Tb, maximum Tb, recovery Tb, and time to reach maximum. They conform to the results from nonlinear mixed model that was shown above. It implies that the nonlinear mixed bi-logistic model provided an appropriate way to describe heifer's Tb during heat stress. However, several parameters, such as rates constants and inflection points of challenge and recovery cannot be estimated using simplistic approach. To gain more important information in decision making during heat stress, a nonlinear mixed bi-logistic model would be preferred to the simplistic approach.

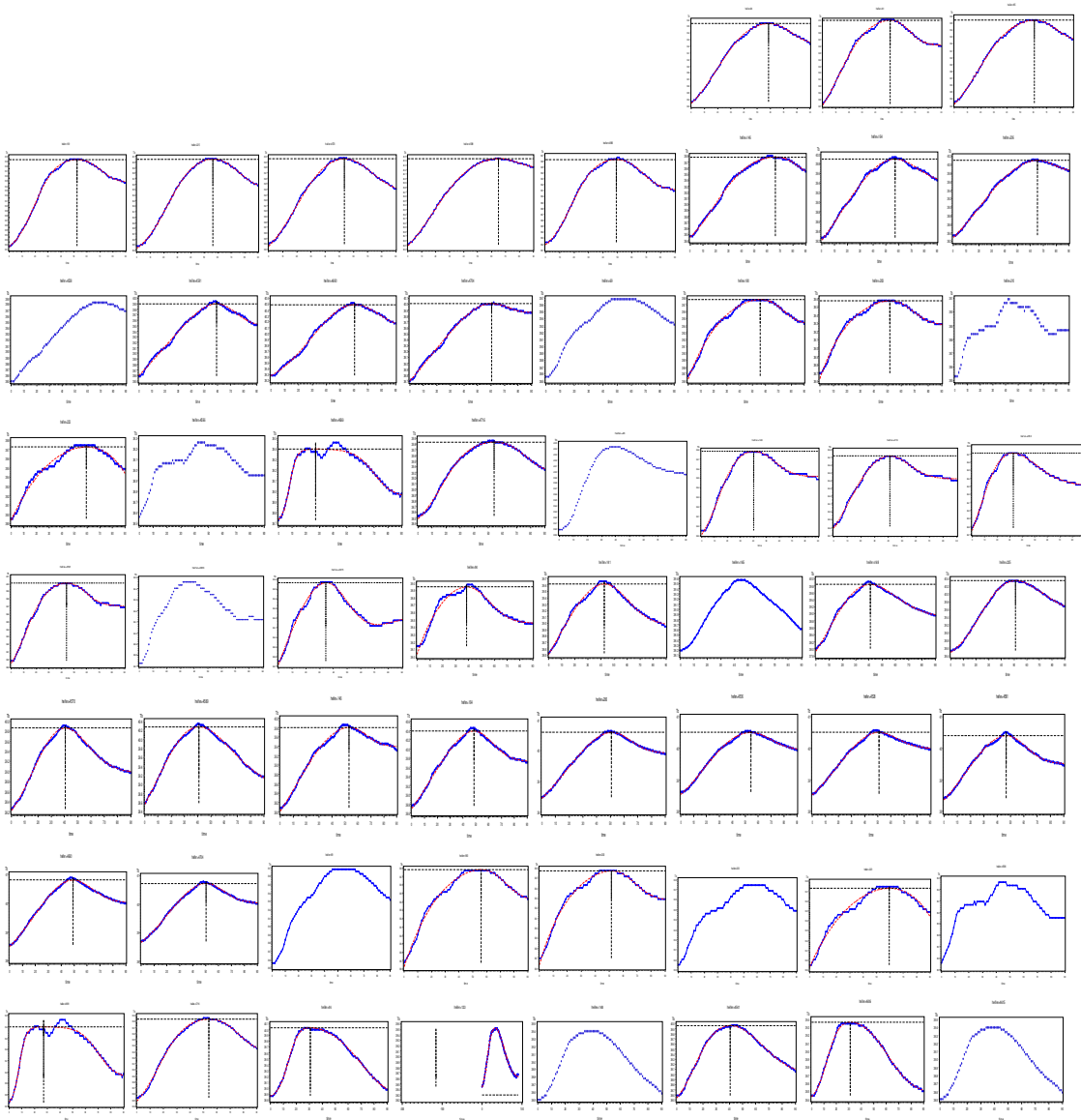
Table 6 The simplistic analysis on the Tb from feedlot heifers while being held in a working chute after being moved from their pens to the processing facility. Data were extracted from the individual heifers' body temperature files.

Parameter	Estimate \pm SE	Significance
Initial Body Temperature, °C	38.40 \pm 0.023	Pen, Date
Maximum Body Temperature, °C	39.74 \pm 0.027	Pen, Date
Recovery Body Temperature, °C	38.81 \pm 0.021	Pen, Date
Time to Maximum Temperature, min	37.6 \pm 0.62	Pen, Date

3.6 Comparisons of R and SAS results

The model fitting and parameter estimation procedure can also be performed using SAS procedure PROC NLMIXED, etc. Both estimates and the corresponding standard errors for all 7 parameters and 2 derived parameters can be obtained. SAS produced similar predictions in most of the heifers. However, one drawback for using SAS is that more cases (12 cases) failed to converge in SAS (Figure 7) than in R (Figure 5) due to the convergence criteria in SAS. Therefore, researchers would choose to use either R or SAS depending on the main objectives. R would be preferred for purpose of prediction; SAS would be preferred for purpose of parameter estimation.

Figure 7 Multiple plots for raw Tb and predicted Tb with estimated maximum body temperature, $\max Tb$, and time to reach maximum body temperature, $t_{\max Tb}$. Blue dashed line -Raw data; Red line -Fitted data.



4. CONCLUSION

An additive bi-logistic model can be used to describe the dynamics of Tb during moving and handling of heifers. The additive nonlinear bi-logistic mixed model with seven parameters,

upper asymptote body temperature, initial body temperature, baseline body temperature for recovery, challenge and recovery rate constants and challenge and recovery inflection points, described the overall moving event in which both challenge and recovery were included. Regardless of random structures, treatment effects were necessary in the mixed model. Comparison of random effects favored the grouped heifer/day (nested) random structure over the heifer-day event (crossed) structure. Day and pen are two important factors that influence a heifer's Tb during stages of thermal challenge and recovery. Day-pen interactions occur in all parameters except the recovery inflection point. Characteristics of heifers moved from pens closer to the work station tend to be similar and depend on the day. Heifers moved the farthest on a hot day have a higher upper asymptote Tb and more rapid change in the increasing Tb than on a cool day. Although interaction effects from factors pen and day were found in initial Tb, baseline Tb for recovery, recovery rate constant, and challenge inflection point, they may be caused by the unusual values in pen 4 or 8. This implies other sources of variation, such as an animal's behavior, weight, and health history are present. Consequently, more care is required when moving animals larger distances during hot summer days. Further study of distance and heifer characteristics during thermally challenging conditions provide insight into the dynamics of heat stress.

5. SUMMARY

Heat stress can be a serious problem for animals in hot conditions. Understanding when and how heat stress happens would help to provide better ways to improve an animal's well being. Body temperature can be used to describe an animals' thermo-regulatory response to the environment. One way to model the process of handling and moving

animals during thermal challenge and recovery in summer is to assume the processes are additive and utilize an additive bi-logistic model. The model has seven parameters, initial body temperature, heat challenge rate constant, upper asymptote for body temperature, challenge inflection point, baseline for recovery, recovery rate constant, and recovery inflection point.

In this study, mixed additive bi-logistic models with and without treatment effects using a nested random structure or a crossed random structure were compared. The mixed additive bi-logistic model using a diagonal nested random structure with treatments was preferred. Treatment factors were significant for all parameters except the recovery inflection point. Significant pen and day interactions were found for the other six parameters. During a hot environment, heifers in pens farthest from the work station have a higher upper asymptote T_b and more rapid change in the increasing T_b . Although interaction effects from factors pen and day were found in other four parameters (initial T_b , baseline T_b for recovery, recovery rate constant, and challenge inflection point), they may come from some other sources of variation, such as an animal's behavior, weight, and health history, in some pens.

The mixed additive bi-logistic model with nested random effect structure is helpful for predicting the change in heifer T_b during moving events. Moving cattle during a hot day or for a relatively larger distance in the summer produces heat stress and may produce economic loss. Besides day and pen, a heifer's condition, such as weight and health, needs to be considered when developing management strategies. However, moving

distances and environment are two of the most important factors that need to be considered when moving animals.

6. FUTURE STUDY

Additive bi-logistic model provides a way to describe heifer's body temperature during heat stress. However, a drawback for this model observed in Figure 5 is that the predicted maximum T_b cannot hit the peak in the raw data. In other words, body temperature produced by additive bi-logistic model was underestimated. To get a better estimation, the model needs to be improved.

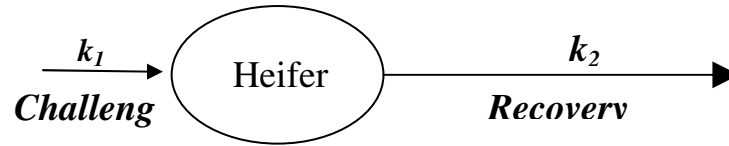
One attempt to improve the model is to make the following assumptions.

- When an animal absorbs heat during thermal challenge, the relative rate of heat accumulation is not constant but is proportional to the amount of heat remaining from the upper asymptote T_b .
- When an animal eliminates heat during thermal recovery, the relative recovery rate is proportional to the amount of heat above the baseline.

Then, the relationship, shown in Figure 8, can be represented in (9). Differential equations in (9) consist of a compartmental model.

$$\begin{aligned} \frac{dTb_{challenge}}{dt} &= k_1(\alpha - Tb_{challenge})(Tb_{challenge} - \beta) \\ \frac{dTb}{dt} &= k_1(\alpha - Tb_{challenge})(Tb_{challenge} - \beta) - k_2(Tb - \delta)(Tb - \beta) \end{aligned} \quad (9)$$

Figure 8 Diagram of a compartment model showing the heat challenge and recovery on a heifer during heat stress



It is interested to try to improve the additive bi-logistic model by solving (9). The solution would be a second order compartment bi-logistic function. Once (9) is solved, the parameter estimation procedure would be performed in the new nonlinear mixed model so that fixed and random effects can be examined and significant interactions on parameters can be estimated and interpreted.

Volterra integral equation was introduced here to solve eqn (9). (Corduneanu. 2008)
Suppose the initial value problem is

$$\frac{dy}{dt} = f(t, y(t)) \quad (10)$$

with initial condition $y_0 = y(0)$,

Take integral on both side,

$$\int_0^t \frac{dy}{ds} ds = \int_0^t f(s, y(s)) ds \quad (11)$$

Then the LHS = $\int_0^t \frac{dy}{ds} ds = y(t) - y(0) = y(t) - y_0$.

Hence, (11) can be written as

$$y(t) = \int_0^t f(s, y(s)) ds + y_0 \quad (12)$$

Equation (12) is a Volterra integral equation for y , and it is entirely equivalent to the initial value problem (10). For some simple cases, (12) can be solved directly by taking

integral on $f(t, y(t))$. However, in our problem (9), it is not easy to solve the RHS of (12) directly by integration. Therefore, we would like to apply iterative procedures, such as Minimum routine method (Thompson, D.E., 1999), Picards Method (Collins, P.J. 2006), Regular Perturbation Method (Shivamoggi, B.K., 2003), to determine the approximate solution.

Minimum routine method is a straightward method for estimating parameters. The main idea is to estimate parameters over ranges to minimize the sum of squared errors. Picards method and Regular perturbation method are two approximation methods.

In Picards method, the integral equation (12) can be solved approximately by fixed-point iteration. Beginning with an initial approximation $\phi_0(t)$, we can generate a sequence of successive approximations $\phi_1(t), \phi_2(t), \dots$, via

$$\phi_{n+1}(t) = \phi_0(t) + \int_0^t f(s, \phi_n(s)) ds \quad (13)$$

$n=0, 1, 2, \dots$

It can be shown that the sequence $\phi_n(t)$ converges to the unique solution (12).

In Regular Perturbation method, we suppose the differential equation is

$$F\left(t, y, \frac{dy}{dt}, \frac{d^2y}{dt^2}, \varepsilon\right) = 0, t \in I \quad (14)$$

where t is the independent variable, I in the interval, and y is the dependent variable. ε is a small parameter smaller than 1. $\varepsilon \ll 1$

Then, the Perturbation series is

$$y(t) = y_0(t) + \varepsilon y_1(t) + \varepsilon^2 y_2(t) + \dots \quad (15)$$

The idea of regular Perturbation method is to assume a solution of (12), where the functions $y_0(t), y_1(t), y_2(t), \dots$ are to be determined by substitution of (15) into (14).

The first few terms of (15) form an approximate solution, named perturbation method. (usually no more than two or three terms were taken.)

Consequently, how to effectively build the compartment bi-logistic function, construct the mixed model, and make interpretation about parameters remain good topics for future study.

7. REFERENCES

- Aitha, N., A.M. Parkhurst, C.N. Lee, P.E. Hillman. 2008. Nonlinear mixed models to evaluate effects of environment conditions, hair coat, and anchor length on body temperature during afternoon milking of holstein cows in Hawaii. Proc. of 20th Annual Kansas State University Conference on Applied Statistics in Agriculture.206-217. Kansas State Univ. Manhattan, Kansas.
- Brent, R. 1973. Algorithms for Minimization without Derivatives. Englewood Cliffs, NJ: Prentice-Hall.
- Brown-Brandl, T.M., R.A. Eigenberg, J.A. Nienaber. 2009. Water spray cooling during handling of feedlot cattle. International Journal of Biometeorology. Published online: <http://www.springerlink.com/content/nm9n417261217624/>

- Corduneanu, C. 2008. Principles of Differential and Integral Equations. AMS Chelsea Publishing. P119-131
- Collins, P.J. 2006. Differential and Integral Equations. Oxford University Press, USA. P181-184
- Davis, M.S., T.L. Mader, S.M. Holt, A.M. Parkhurst. 2003. Strategies to reduce feedlot cattle heat stress: effects on tympanic temperature. *J Anim Sci* 81:649–661
- Kerek, M., A.M. Parkhurst, T.L. Mader. 2003. Using the bi-logistic model to estimate body temperature in feedlot cattle. Proc. of 15th Annual Kansas State University Conference on Applied Statistics in Agriculture. 206-216. Kansas State Univ. Manhattan, Kansas.
- Li, X., A.M. Parkhurst, T.L. Mader. 2009. Comparing experimental designs for A bi-logistic model used to estimate heat stress when moving feedlot cattle. Proc. of 21st Annual Kansas State University Conference on Applied Statistics in Agriculture. 182-197. Kansas State Univ. Manhattan, Kansas.
- Parkhurst, A.M. and T.L. Mader. 2000. Using nonlinear growth curves to estimate heat stress in processing feedlot cattle. Proc. of 12th Annual Kansas State University Conference on Applied Statistics in Agriculture. Manhattan, Kansas.

Pinheiro, J.C. and D.M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer.
New York

SAS Institute Inc. 2010. SAS/STAT® 9.2 User's Guide. Cary, NC: SAS Institute Inc.

Shivamoggi, B.K., 2003. Perturbation methods for differential equations. AMS Chelsea
Publishing. P13-40.

Thompson, D.E., 1999. Design analysis: mathematical modeling of nonlinear systems.
Cambridge University Press. P80

Zhou, M., A.M. Parkhurst, R.A. Eigenberg, J. Nienaber, G.L. Hahn. 2006. Evaluating
nonlinear crossed random effects models for comparing temperature of eating pigs
under different thermal environments. Proc. of 18th Annual Kansas State
University Conference on Applied Statistics in Agriculture. 180-200. Kansas State
Univ. Manhattan, Kansas.