**Metabolic differences in temperamental Brahman cattle can affect productivity**[1,2]

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## Introduction

Many factors may adversely affect the growth and productivity of livestock. These include stressors associated with management practices, such as weaning, handling relative to transportation, and vaccination, that can modulate growth through the production of stress-related hormones (i.e., cortisol, epinephrine, and norepinephrine; Crookshank et al, 1979; Rulofson et al., 1988; Lay et al., 1992; Carrasco and Van de Kar, 2003; Charmandari et al., 2005; Buckham Sporer et al., 2008). As the cost of cattle production continues to increase, it is essential for producers to find ways to decrease input costs in order to increase profit. Temperament is an additional factor that can influence the productivity of cattle. Temperament is defined as the manner in which cattle react to humans or novel environments (Fordyce et al., 1988).

Various methods are used to measure temperament, with the two most commonly used by our laboratories being pen score and exit velocity (see Burdick et al., 2011b for review). Pen score is a subjective method to measure temperament. For this particular measurement, cattle are separated in groups of 3 to 5 animals and their reactivity to a human observer is ranked on a scale of 1, described as calm, docile, and approachable, to 5, described as volatile, very aggressive, and crazy (Hammond et al., 1996). For more information, refer to the BIF Guidelines on pen score. Exit velocity, also referred to as flight speed, is emerging as a more objective measurement of temperament in cattle (Fell et al., 1999; Curley et al., 2006; Müller and Von Keyserlingk, 2006; Vann et al., 2008). Exit velocity (Burrow et al., 1988; Curley et al., 2006) is defined as the rate (in feet/second) at which an animal traverses a specified distance after exiting a squeeze chute. As different aspects of behavior may be measured by different temperament assessment methods (Curley et al., 2006), it is possible that a combined score utilizing multiple methods (i.e. average of pen score and exit velocity to determine a temperament score) may allow more accurate temperament classification. Based on temperament score, cattle can be ranked into temperament groups (i.e., calm, intermediate, and

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temperamental). A previous study from our group reported the heritability of pen score (0.44), exit velocity (0.28), and temperament score (0.41) in Brahman cattle (Loyd et al., 2011).

Several research groups have demonstrated that temperament can negatively affect various production traits, including live weight, average daily gain, dry matter intake, conception rates, milk yield, carcass weight, tenderness, rib fat, and bruising score (Hafez and Lindsay, 1965; Fordyce et al., 1985; Fordyce et al., 1988; Burrow and Dillon, 1997; Breuer et al., 2000; Petherick et al., 2003; Prayaga and Henshall, 2005; King et al., 2006; Müller and von Keyserlingk, 2006; Hoppe et al., 2010; Café et al., 2011). Additionally, cattle temperament has been linked to stress responsiveness. Specifically, cattle that are more temperamental have greater circulating concentrations of the adrenal gland derived stress hormones cortisol and epinephrine (Curley et al., 2006; Burdick et al., 2009), which also markedly affect metabolism. The adrenal glucocorticoid cortisol stimulates the production of glucose from substrates such as lactate, glycerol and amino acids (i.e., gluconeogenesis) in the liver, inhibits the uptake of glucose into adipose tissue, and continuously stimulates the breakdown of muscle protein. The adrenal catecholamine epinephrine (i.e., adrenaline) increases plasma concentration of the energy substrates glucose and non-esterified fatty acids (NEFAs; free fatty acids that are not linked to a glycerol molecule) through stimulating the breakdown of glycogen, the molecule by which glucose is stored, and triglycerides. The inherently greater concentrations of metabolically active stress hormones in temperamental cattle may be the basis for how temperament affects metabolic performance during both stress-free and stressful circumstances. The interaction between temperament and metabolism is one area that has yet to be studied in sufficient detail. Herein, we discuss three studies aimed at elucidating the potential influence of temperament on metabolism.

## Results and Discussion

A study was designed to determine the effect of temperament on the metabolic response of calves to a glucose tolerance test. During a glucose tolerance test, cattle are administered glucose, and the glucose and insulin responses are monitored. The test is utilized to determine the time it takes for insulin to clear the exogenous glucose, as well as to monitor the relationship between insulin and glucose in order to determine insulin sensitivity or insensitivity. While the test has been utilized in humans as a test for type 2 diabetes, it has also been used in the dairy cattle industry in order to understand the influence of stress and nutritional state on dairy cow milking traits (Lemosquet and Faverdin, 2001). Cortisol plays a major role in glucose metabolism, as discussed above; therefore, it has been hypothesized that the greater basal concentrations of cortisol secreted by temperamental cattle may alter glucose clearance and the insulin response to a glucose tolerance test. For this study, temperament score was determined for 36 Brahman heifers, from which the 6 most temperamental and 6 most calm heifers were utilized for a glucose tolerance test (Bradbury, 2011). During the 90-min period following placement of a jugular catheter and prior to the onset of the glucose tolerance test, temperamental heifers maintained greater plasma concentrations of glucose and cortisol compared to calm heifers (*P* < 0.01 for both glucose and cortisol). Following administration of the glucose bolus, temperamental heifers maintained greater concentrations of cortisol (*P* = 0.03) and glucose (*P* < 0.01; Figure 1) compared to calm heifers. Additionally, there was a time by temperament interaction such that calm heifers had significantly greater concentrations of insulin than temperamental heifers from 10 to 60 min following administration of the glucose tolerance test (*P* < 0.01; Figure 2). Overall, time to peak insulin concentration, glucose half-life concentration, and glucose half-life time were all greater in temperamental heifers than calm heifers (*P* < 0.01 for all variables). Yet, peak insulin concentration was greater in calm than temperamental heifers (*P* = 0.04). In general, these data demonstrate that temperamental cattle have greater concentrations of cortisol, which remain elevated during periods of stress. Additionally, these data demonstrate that calm heifers were able to clear glucose at a much faster rate than temperamental heifers. Insulin is responsible for increasing the uptake of glucose into adipose and muscle tissue (Hocquette and Abe, 2000). Therefore, temperamental cattle may be more resistant to insulin compared to calm cattle, thus decreasing the amount of glucose that the cattle can absorb and store in muscle, fat, and the liver. As temperament modifies metabolic regulatory responses in heifers, this altered metabolism may partially explain their decreased productivity.

In addition to influencing metabolism, cortisol can also influence the immune response. Elevated cortisol concentration, induced by acute stress (i.e., exposure to a stressor for a short duration of time) is not necessarily detrimental to an animal's health, and may actually enhance immune functions. However, chronic stress, or stress extended over a prolonged period of time, can be detrimental to the health and well-being of livestock. Therefore, it was hypothesized that temperamental cattle would have an altered response to an immune stimulus compared to calm cattle. To test this hypothesis, a study was conducted that utilized calm (n = 8), intermediate (n = 8), and temperamental (n = 8) Brahman bulls, selected based on temperament score from a pool of 60 bulls, in order to determine their response to an immune challenge (i.e., lipopolysaccharide, LPS, a component of the cell wall of gram negative bacteria such as *E. coli*; Burdick et al., 2011a). Prior to administration of LPS, temperamental bulls had greater rectal temperature (*P* < 0.01), and greater cortisol (*P* < 0.01) and epinephrine concentrations (*P* < 0.01). Following administration of LPS, rectal temperature increased in all bulls, with temperamental bulls producing the smallest increase in rectal temperature (i.e., relative to baseline values) compared to calm and intermediate bulls (*P* < 0.01). Sickness behaviors, measured on a scale of 1 (normal maintenance behaviors) to 5 (head distended and lying on side with labored breathing) were also lower in temperamental bulls than intermediate and calm bulls (*P* < 0.01; Figure 3). Therefore, these data suggest that temperamental cattle may display limited behavioral signs of illness, which may prevent proper medical intervention, and increase the risk of transferring pathogens to healthy, calmer cattle. While absolute cortisol concentrations were not different between temperament groups following LPS administration (*P* = 0.80), the change in cortisol relative to baseline concentration (i.e., measured from -2 to 0 h prior to LPS administration) was greater in calm and intermediate bulls than temperamental bulls (*P* < 0.01; Figure 4). The greater cortisol concentrations in temperamental bulls prior to LPS administration may have resulted in the blunted cortisol response observed following LPS administration, which is similar to the results observed by Curley et al. (2008) in which cortisol secretion was stimulated by exogenous administration of adrenocorticotropic hormone (ACTH). Concentrations of plasma epinephrine also remained elevated in temperamental bulls following administration of LPS ($P < 0.05$; Figure 5). In summary, differences exist in the physiological (i.e., rectal temperature and sickness behavior) as well as endocrine (i.e., cortisol and epinephrine) responses of temperamental cattle to an LPS challenge.

The immune system has a high energy demand when activated. It has been estimated that to increase body temperature 1$^{o}$C an animal must increase its metabolic rate by 10 to 13% (Carroll and Forsberg, 2007). Aside from increasing body temperature, there are additional energy requirements for other immune functions, such as the production of antibodies and acute phase proteins. As demonstrated in the first study, temperament can influence metabolic parameters, including glucose and insulin. These data, together with the immune response data, led our group to hypothesize that differences observed in response to an immune challenge are due to differences in energy availability between calm and temperamental cattle.

To test this hypothesis samples collected during the LPS challenge described above were analyzed for various metabolic parameters. Results from this study indicate that temperamental bulls had an altered metabolic response compared to intermediate and calm bulls (Carroll et al., 2011). Specifically, there was a time by temperament interaction ($P < 0.01$) such that concentrations of glucose increased in response to LPS challenge in calm and intermediate bulls, but there was no increase in glucose concentration observed in temperamental bulls (Figure 6). Additionally, insulin, released in response to increasing blood glucose concentrations, was greater in calm bulls than intermediate and temperamental bulls following administration of LPS ($P < 0.01$). Due to the high concentrations of glucose and insulin observed in calm bulls, it is possible that the calm bulls became insulin resistant, and therefore were unable to properly regulate the uptake of glucose from the circulation and into tissues that required it, which may partially explain the greater amount of sickness behaviors observed in calm bulls. Studies performed in the 1930s and described by Long et al. (1940) found that removal of the adrenal gland, and subsequent decreases in cortisol, caused a decrease in glucose concentrations and a decrease in the ability to store glucose as glycogen in the liver and muscle. It is possible that greater cortisol concentrations observed in the temperamental bulls may make them more resistant to cortisol, as suggested earlier, which may have reduced their subsequent glucose and insulin responsiveness following LPS administration. This is supported by the initial study described above, in which temperamental heifers failed to produce an insulin response to a glucose tolerance test, while maintaining greater concentrations of cortisol compared to calm heifers (Bradbury, 2011).

Temperament also influenced the availability of NEFAs. Specifically, temperamental bulls maintained greater concentrations of NEFA both prior to and following administration of LPS ($P < 0.01$; Figure 7). A greater concentration of NEFA supports a previous report which found that temperamental cattle fail to deposit adequate amounts of fat, and suggests an influence of temperament on adiposity, or fat content (Nkrumah et al., 2007). We concluded that temperamental cattle were utilizing NEFA for energy in the presence of low glucose concentrations, thus preventing the incorporation of fatty acids into triglycerides inside fat cells. It is interesting to note that greater concentrations of NEFA have been linked to insulin resistance (Lam et al., 2003). Additionally, NEFA concentrations were negatively associated with insulin and glucose concentrations during the LPS challenge, suggesting a negative relationship between insulin and NEFA concentrations. Temperamental cattle also had lower concentrations of blood urea nitrogen (BUN), an indicator of protein break down ($P = 0.01$). This suggests that temperamental bulls did not have to break down muscle protein in order to provide energy during the immune challenge, as did intermediate and calm bulls. Collectively, these data suggest that temperamental cattle may be utilizing NEFA rather than glucose for energy, which may have influenced their response to the LPS challenge.

Together, these data suggest that clear metabolic differences exist between calm and temperamental Brahman calves. The decreased ability to utilize glucose, likely due to a high concentration of cortisol, supports the potential for temperamental cattle to utilize an alternate source of energy when glucose concentrations are low. Therefore, it is likely that temperamental cattle utilize free fatty acids, resulting from the continuous lipolysis of adipose tissue, to fuel tissues and organs that can utilize other energy sources rather than glucose. As temperamental cattle do not deposit fat at the same rate as do calm cattle (Nkrumah et al., 2007), producers may want to feed temperamental cattle differently as they may not reach the same quality grade as calmer cattle. These data go against treating ‘all cattle the same’, as alternative management for temperamental cattle (e.g. not implanting due to the likelihood of decreased fat deposition by temperamental cattle) may decrease input costs. Future research by our research team is focused on determining if alternative management strategies for calm versus temperamental cattle can increase profitability through reducing costs.

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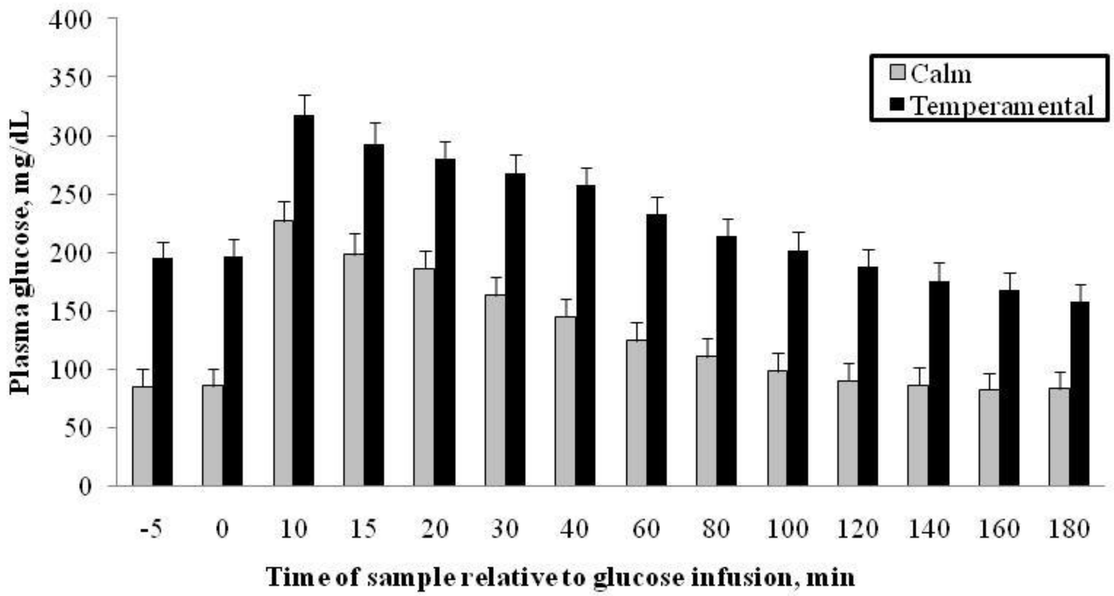


**Figure 1.** Plasma glucose response to administration of a glucose tolerance test in calm and temperamental Brahman heifers (Bradbury, 2011).

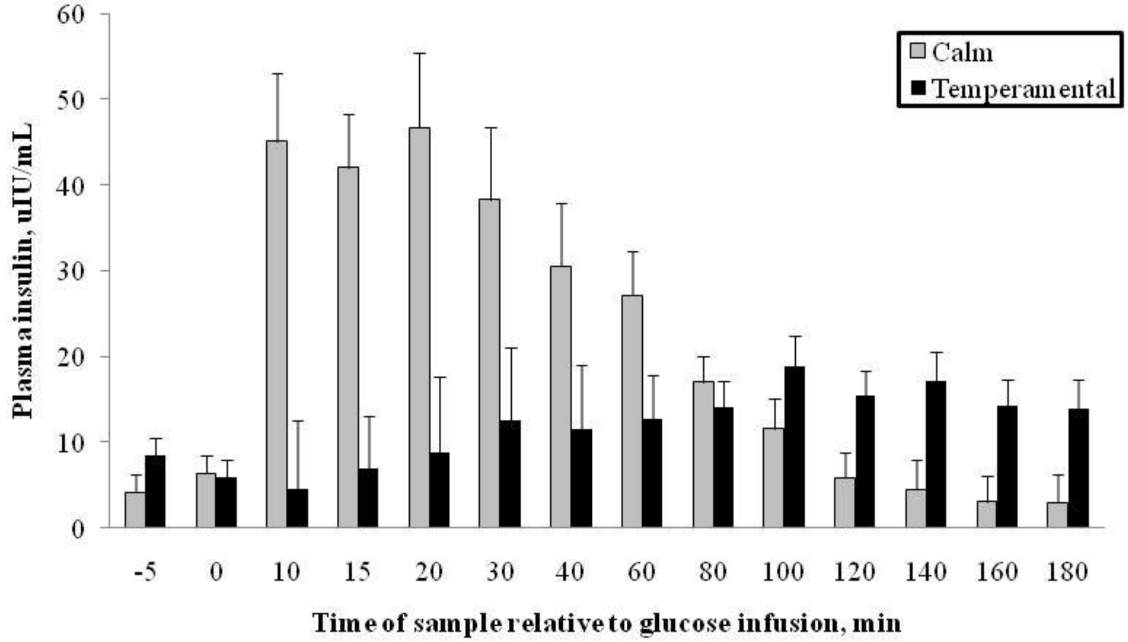


**Figure 2.** The insulin response to administration of a glucose tolerance test in calm and temperamental Brahman heifers (Bradbury, 2011).

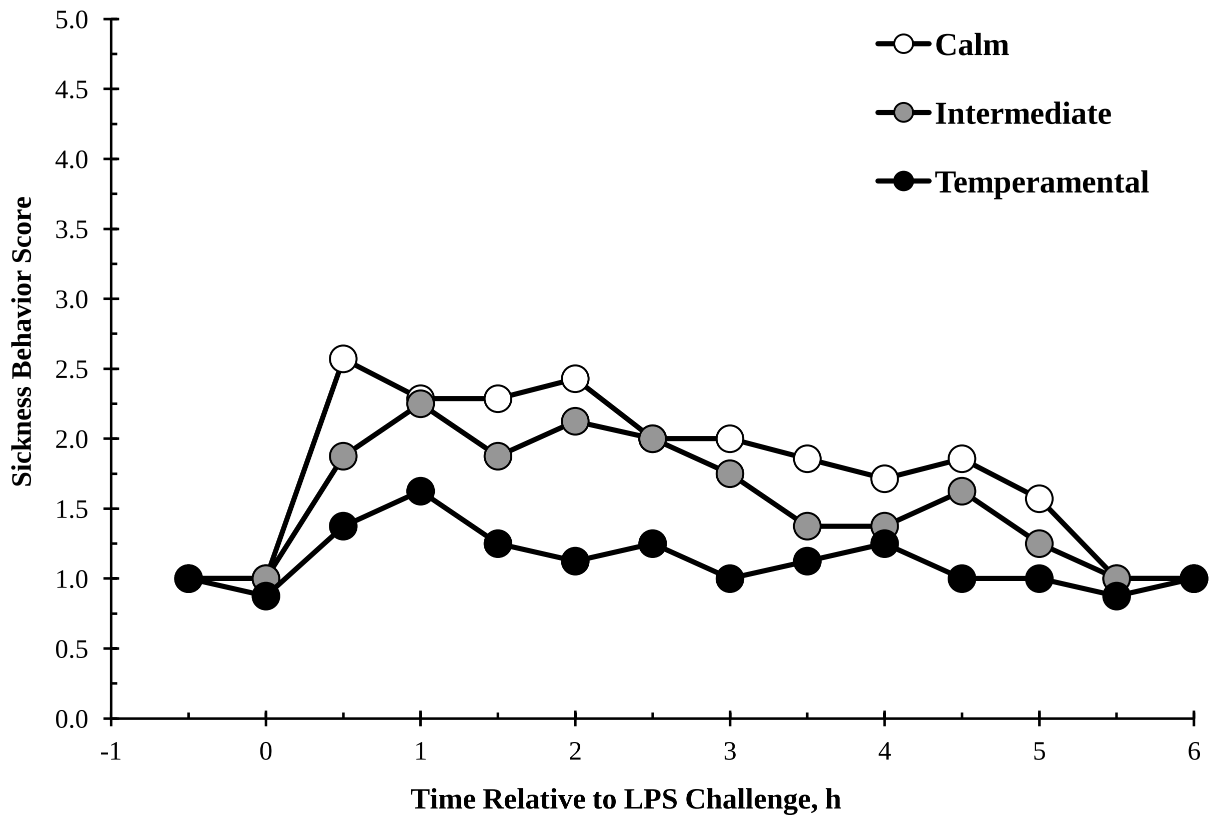

**Figure 3.** Sickness behavior response to administration of lipopolysaccharide (LPS) in calm, intermediate, and temperamental Brahman bulls (Burdick et al., 2011a).

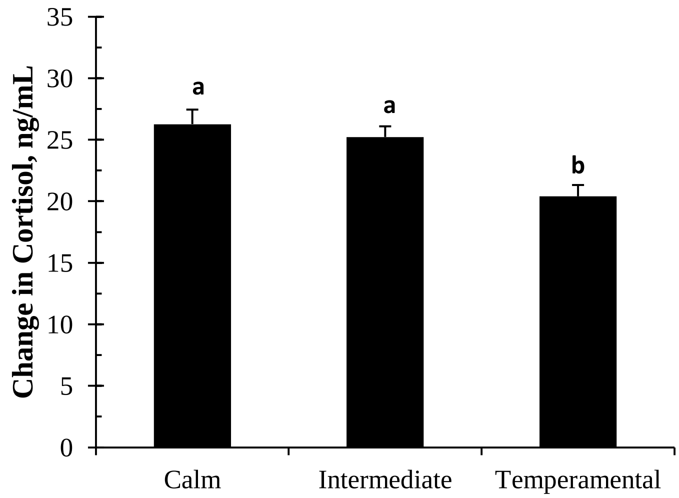


**Figure 4.** Average change in serum cortisol concentration (relative to an average of baseline cortisol concentrations) following administration of lipopolysaccharide (LPS) in calm, intermediate, and temperamental Brahman bulls (Burdick et al., 2011a). Unlike superscripts represent differences between temperament groups ($P < 0.05$).

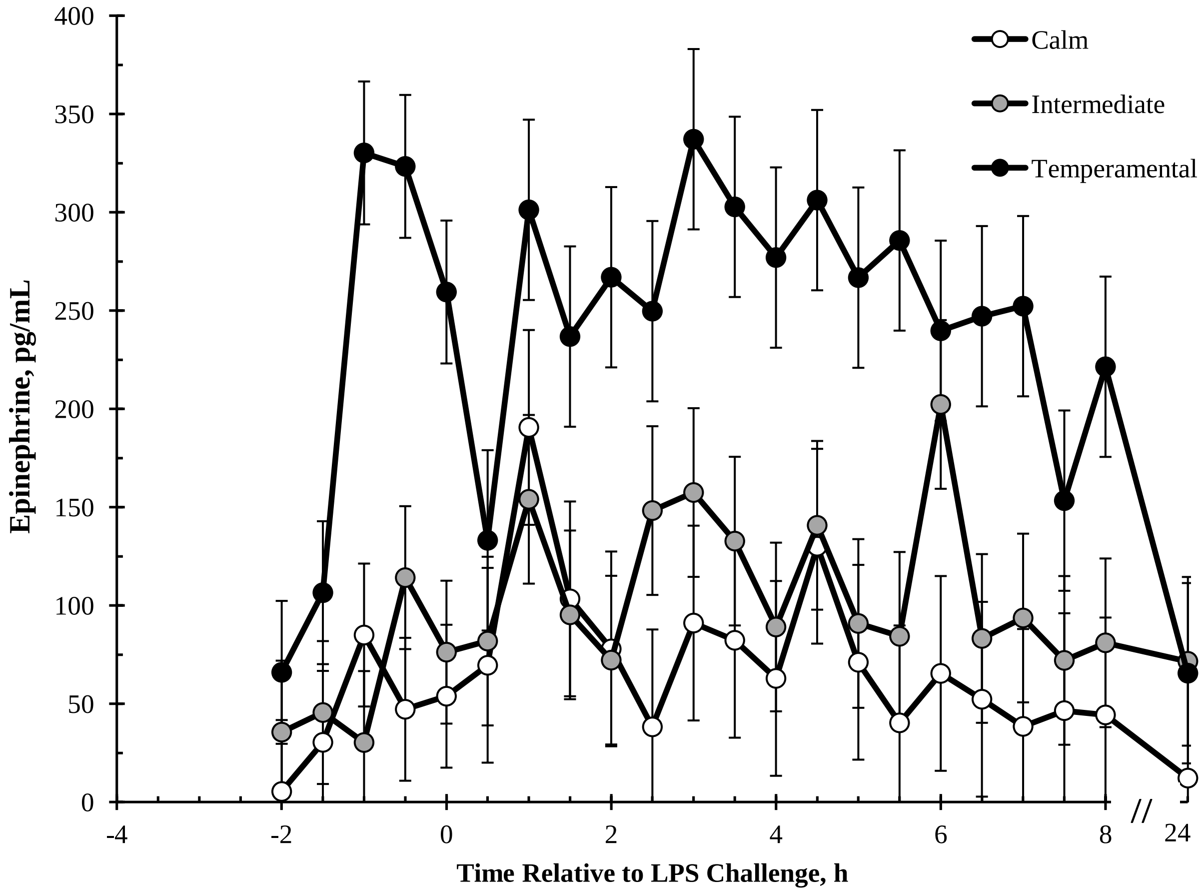


**Figure 5**. Plasma epinephrine response to administration of lipopolysaccharide (LPS) in calm, intermediate, and temperamental Brahman bulls (Burdick et al., 2011a).

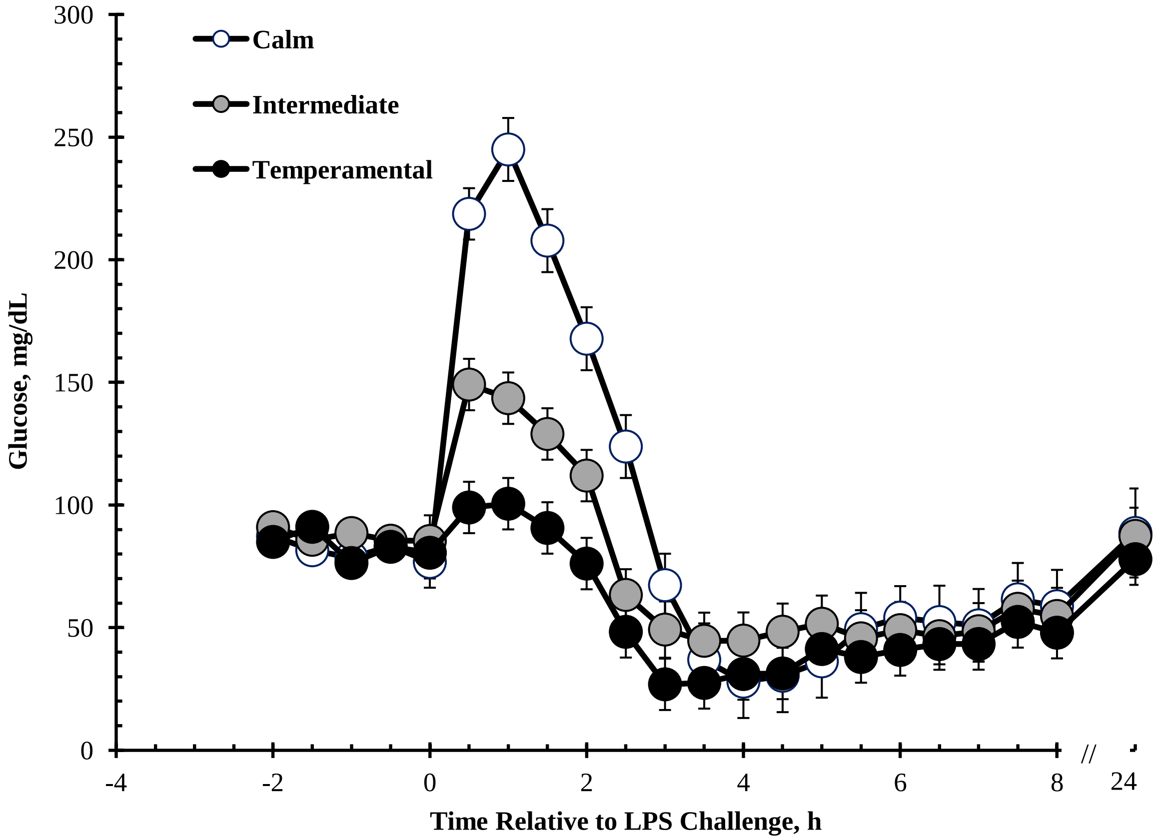


**Figure 6.** Serum glucose response to administration of lipopolysaccharide (LPS) in calm, intermediate, and temperamental Brahman bulls (Carroll et al., 2011).

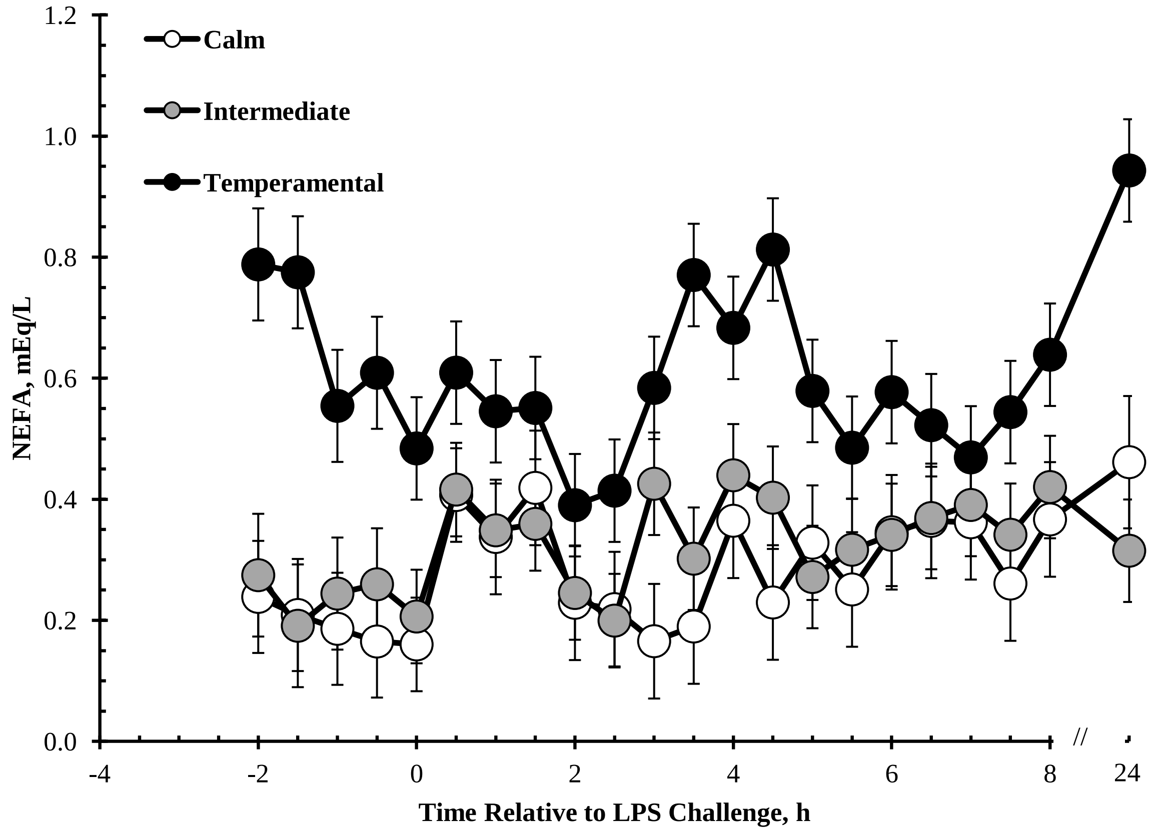


**Figure 7.** Serum non-esterified fatty acid (NEFA) response to administration of lipopolysaccharide (LPS) in calm, intermediate, and temperamental Brahman bulls (Carroll et al., 2011).

# Metabolic Differences in Temperamental Brahman Cattle Can Affect Productivity

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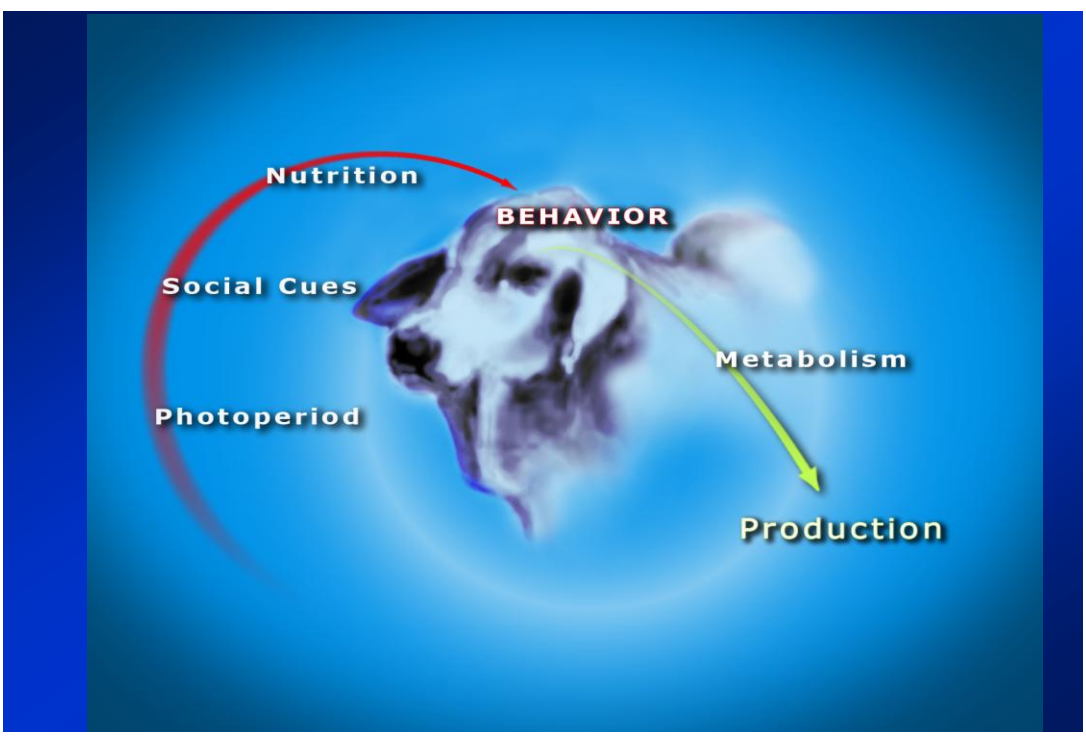


# Temperament

- Temperament in cattle can be defined as the response of an animal to being handled by a human (Fordyce et al., 1982). It is assessed by  the way that cattle react to human handling and interaction.

- Temperament and cattle management
  - Human interaction is inevitable.
  - Destructive to themselves, facilities, other animals.

- Cattle temperament can influence many areas of production including reproduction, immunity, and carcass traits.

- Petherick et al. (2009) reported that fear response can be reduced with proper human handling and association with positive events.
    - Scores re-evaluated over time did not change, even though fear was sometimes lessened.

- Temperament is predominately innate and heritable in *Bos taurus* cattle (Gauly et al., 2001).

- Heritability of pen score (0.44), exit velocity (0.28), and temperament score (0.41) has been reported in Brahman cattle (Loyd et al., 2011).

## Temperament

- Cattle that are more temperamental:
  - Tend to have significantly lower body weights than more calm animals (Tulloh, 2004).
  - Lower BCS, carcass and slaughter weights, dressing percentage (Burrow and Dillon, 1997; Petherick et al., 2002)
  - Lower ADG and higher shear force values (del Campo et al., 2010)
  - Decreased tenderness at slaughter (King et al., 2006)

# Temperament

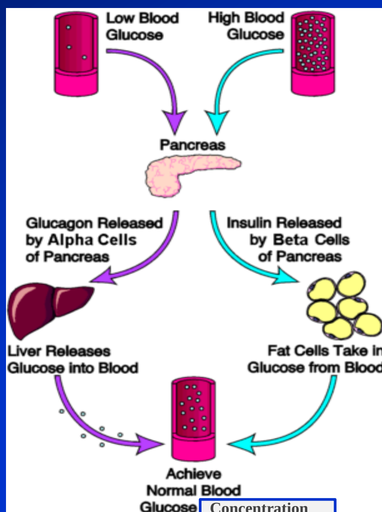
- Animals of different temperaments have diverse functional characteristics of their HPA axis and therefore react to stress differently (Curley et al., 2008).
- Cattle that are more excitable have greater concentrations of stress hormones such as cortisol and epinephrine than calm cattle, which is correlated to temperament.

  (King et al., 2006; Curley et al., 2006, 2008).
- Cortisol (a glucocorticoid) is known as the hormone of stress.

# Glucocorticoids

- Glucocorticoids are steroid hormones that bind to the glucocorticoid receptor, which is present in most animal cells.
- Cortisol is the most important glucocorticoid in cattle.
- Metabolism:
  - Inhibits the uptake of glucose into adipose and muscle tissue.
  - Stimulation of gluconeogenesis in the liver.
  - Stimulates fat breakdown in adipose tissue.

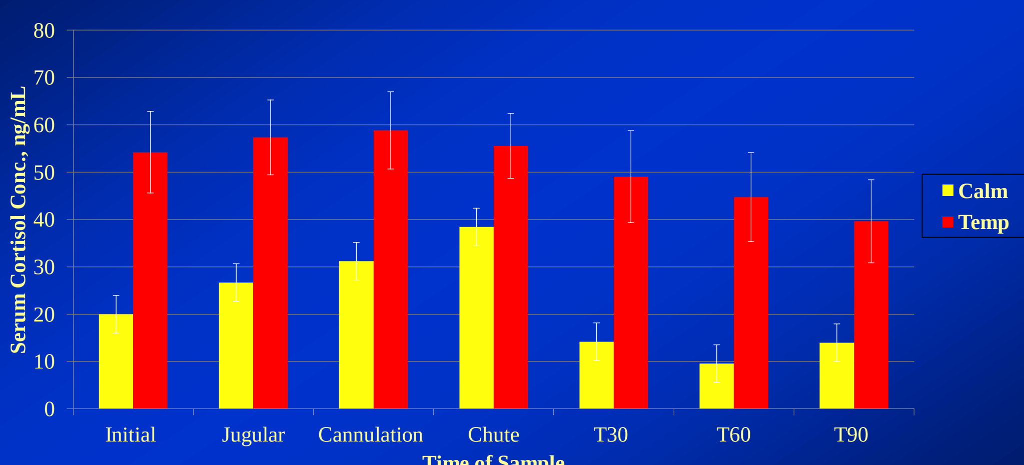
# Glucose Homeostasis



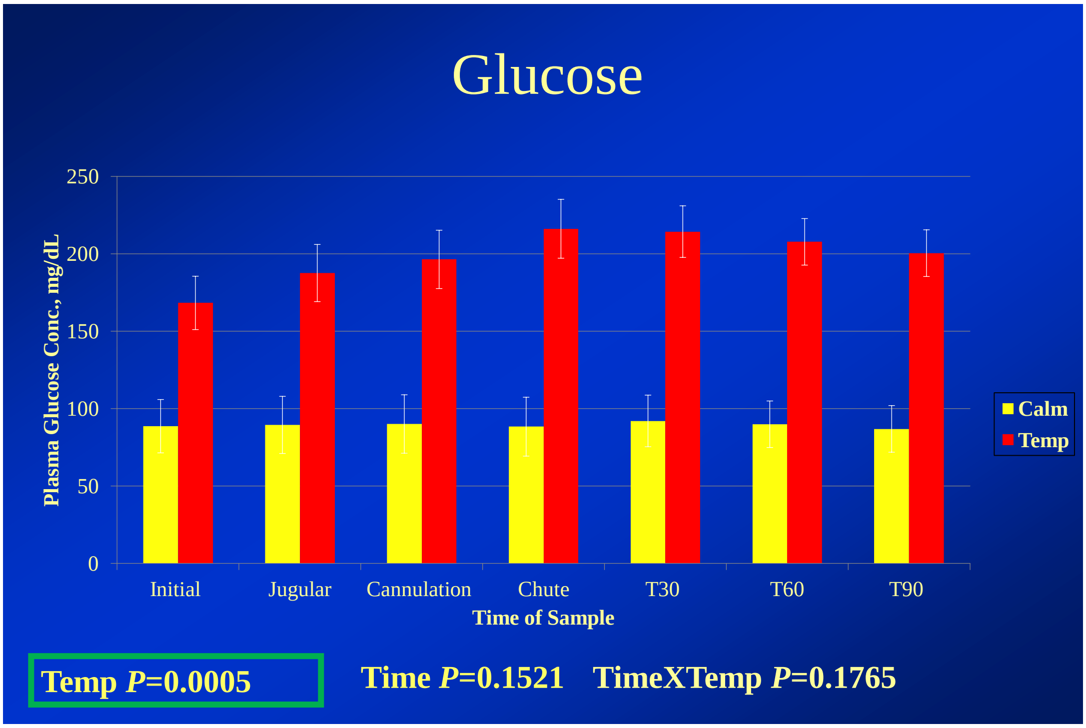

Modified from gettingstronger.org

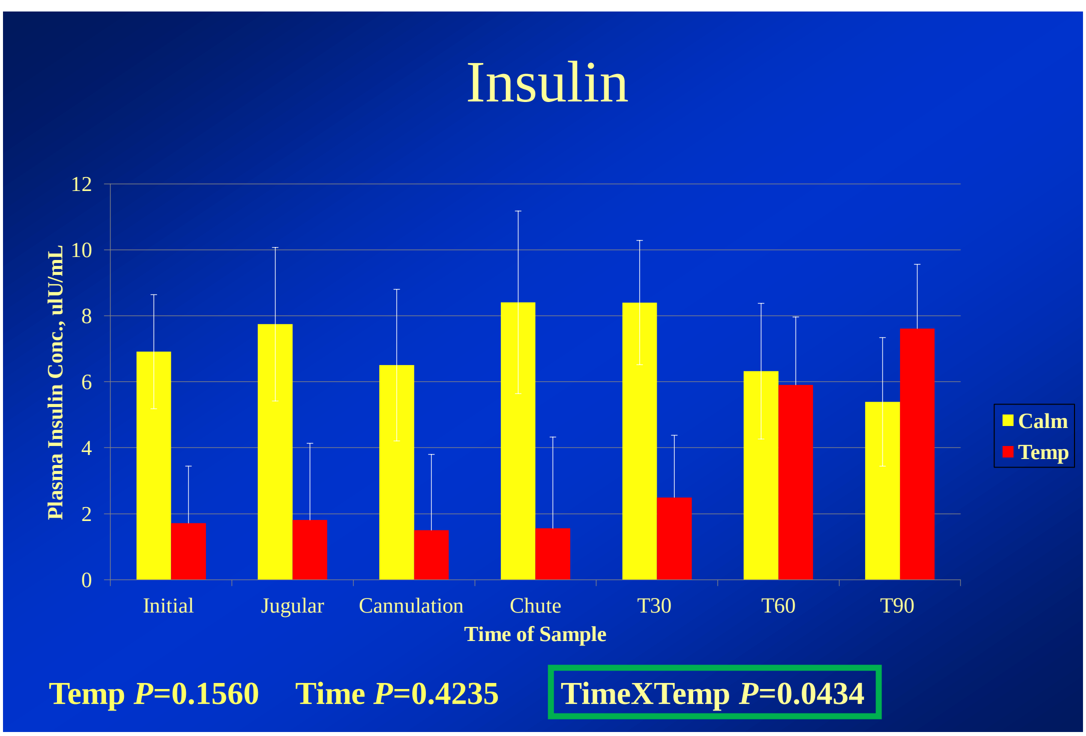
- ❑ Glucose is the most important cellular energy source.
- ❑ Insulin is a metabolic hormone that regulates the concentration of glucose in the blood.

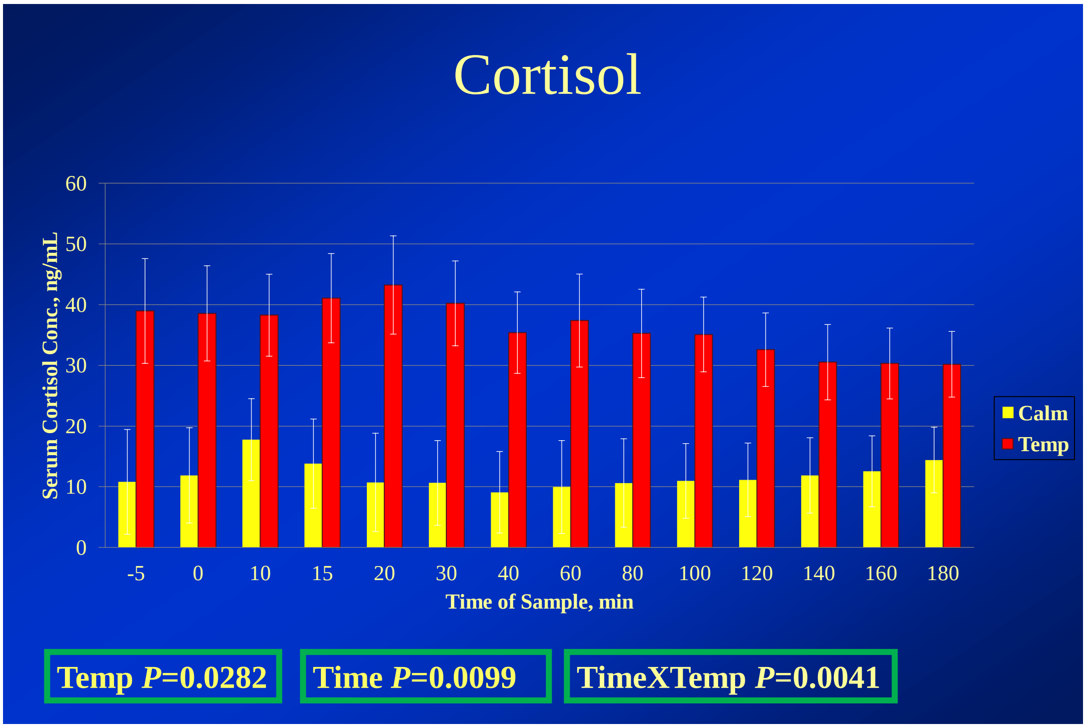
# Cortisol

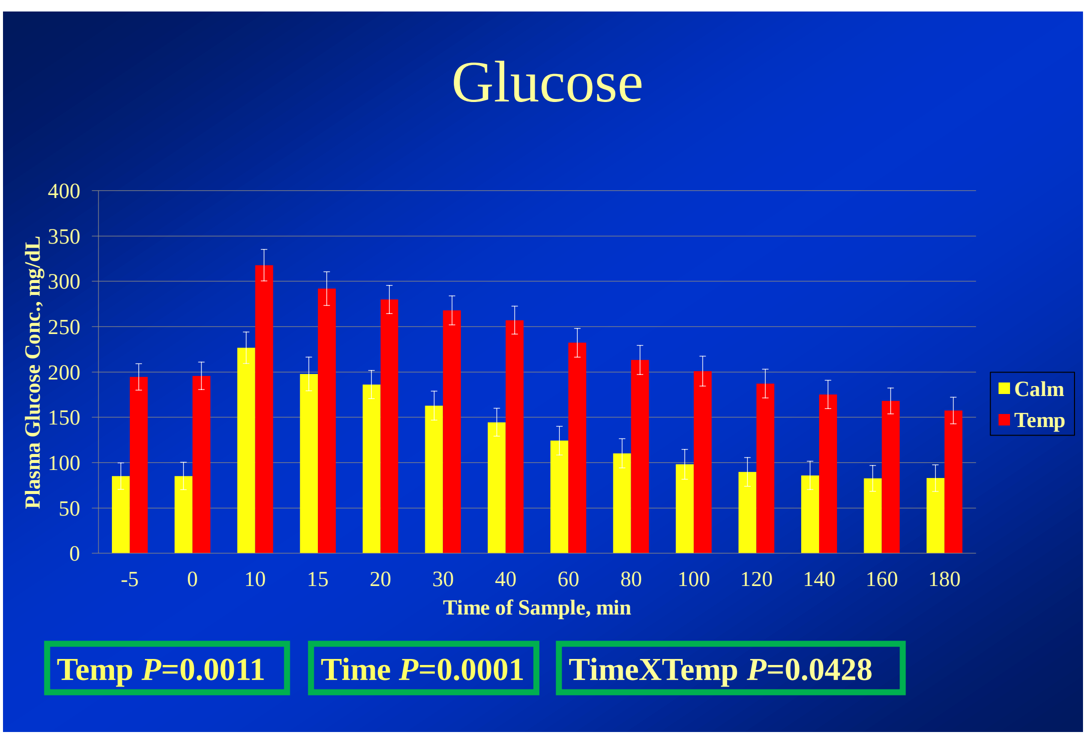



**Temp *P*=0.0238** **Time *P*=0.0002** **TimeXTemp *P*=0.2359**

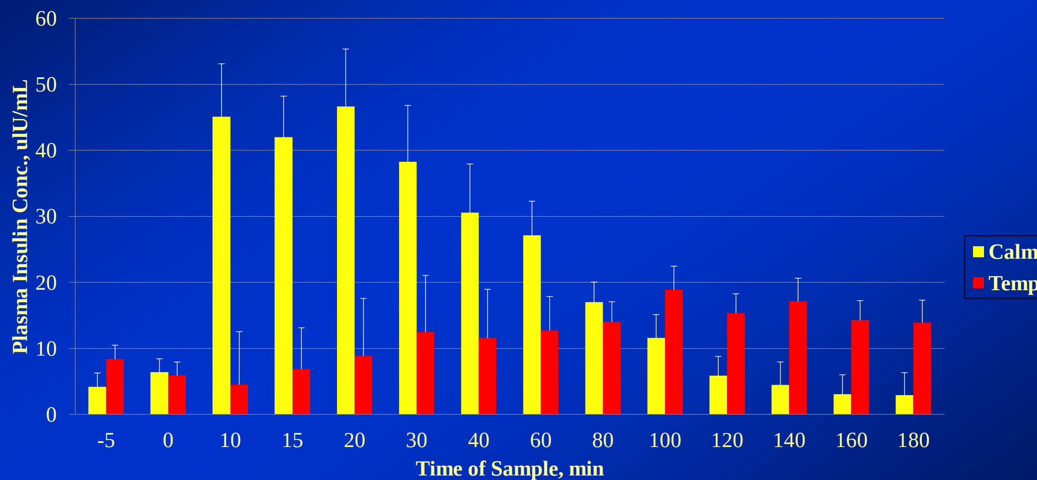

Glucose
Plasma Glucose Conc., mg/dL
250
200
150
100
50
0
Initial
Jugular
Cannulation
Chute
T30
T60
T90
Time of Sample
Calm
Temp
Temp P=0.0005
Time P=0.1521
TimeXTemp P=0.1765


Insulin
Plasma Insulin Conc., uIU/mL
12
10
8
6
4
2
0
Initial
Jugular
Cannulation
Chute
T30
T60
T90
Time of Sample
Calm
Temp
Temp P=0.1560
Time P=0.4235
TimeXTemp P=0.0434


Cortisol
Serum Cortisol Conc., ng/mL
0
10
20
30
40
50
60
-5
0
10
15
20
30
40
60
80
100
120
140
160
180
Time of Sample, min
Calm
Temp
Temp P=0.0282
Time P=0.0099
TimeXTemp P=0.0041


Glucose
Plasma Glucose Conc., mg/dL
0
50
100
150
200
250
300
350
400
-5
0
10
15
20
30
40
60
80
100
120
140
160
180
Time of Sample, min
Calm
Temp
Temp P=0.0011
Time P=0.0001
TimeXTemp P=0.0428

# Insulin



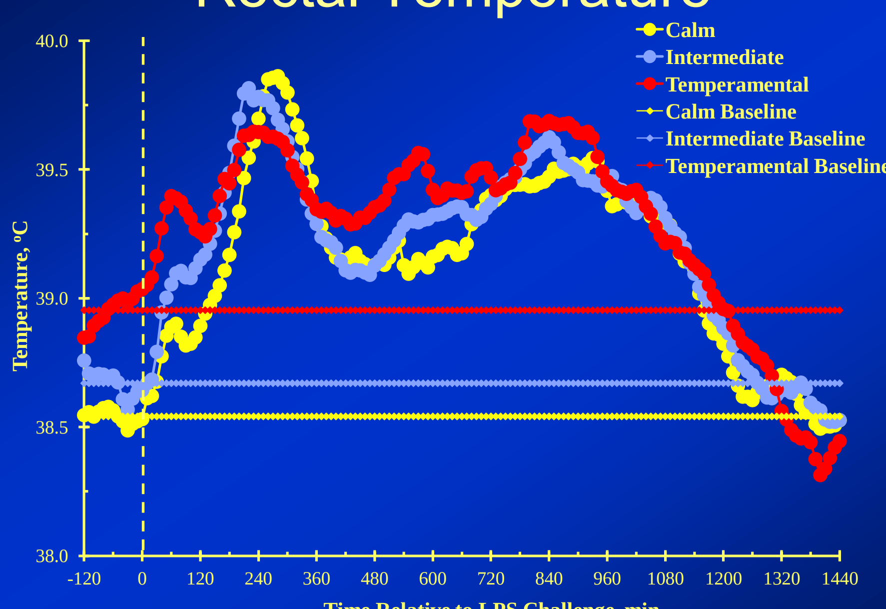

**Temp *P*=0.0793** **Time *P*=0.0022** **TimeXTemp *P*=0.0001**

# Conclusions

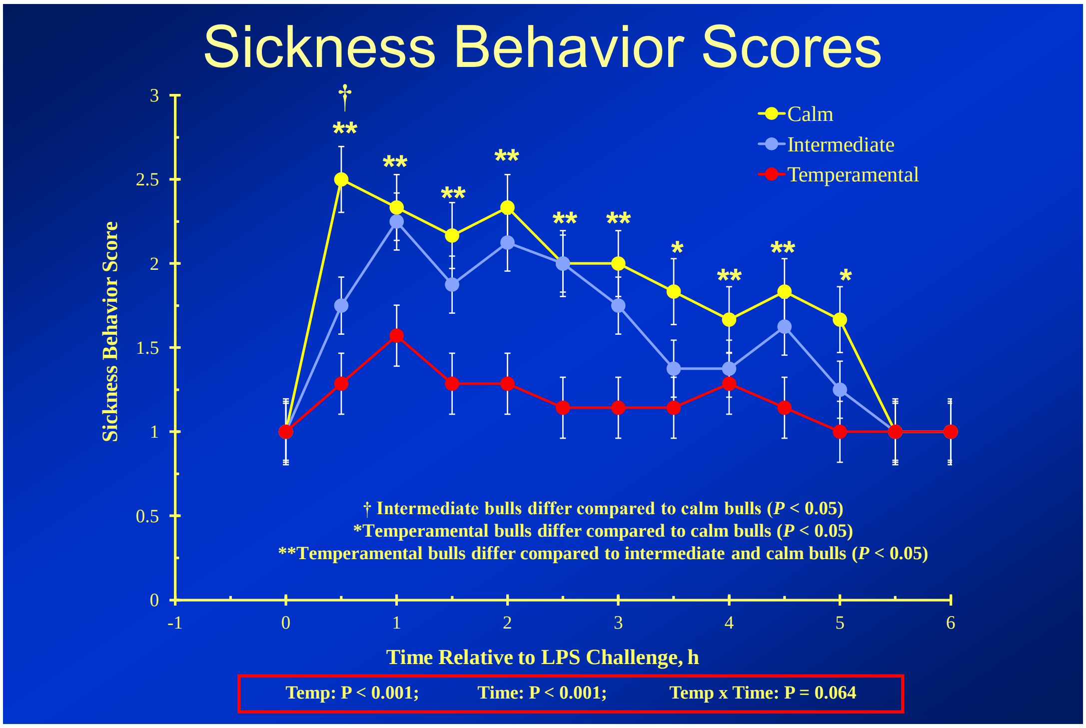
- ❑ Temperamental cattle have greater concentrations of cortisol, which remain elevated when stressed.
- ❑ Temperamental cattle have higher concentrations of glucose in their blood.
- ❑ Temperament modifies metabolic regulatory responses in heifers and this altered metabolism of temperamental cattle may partially explain their decreased productivity.

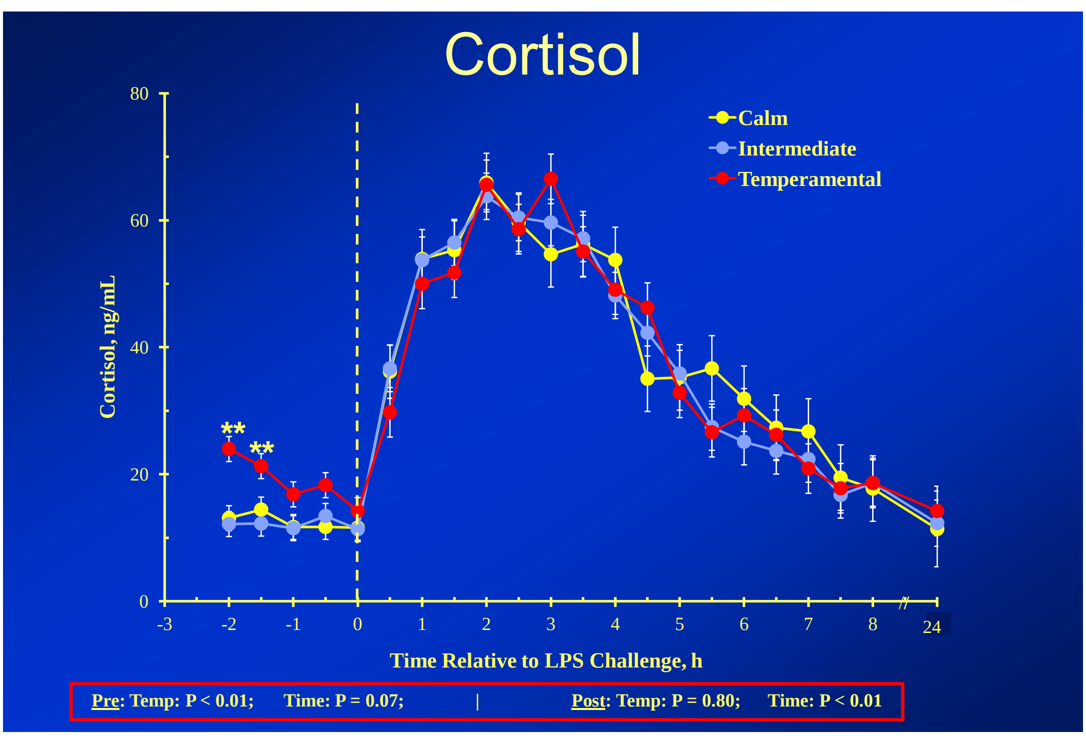
# How do temperamental cattle respond to a disease challenge?

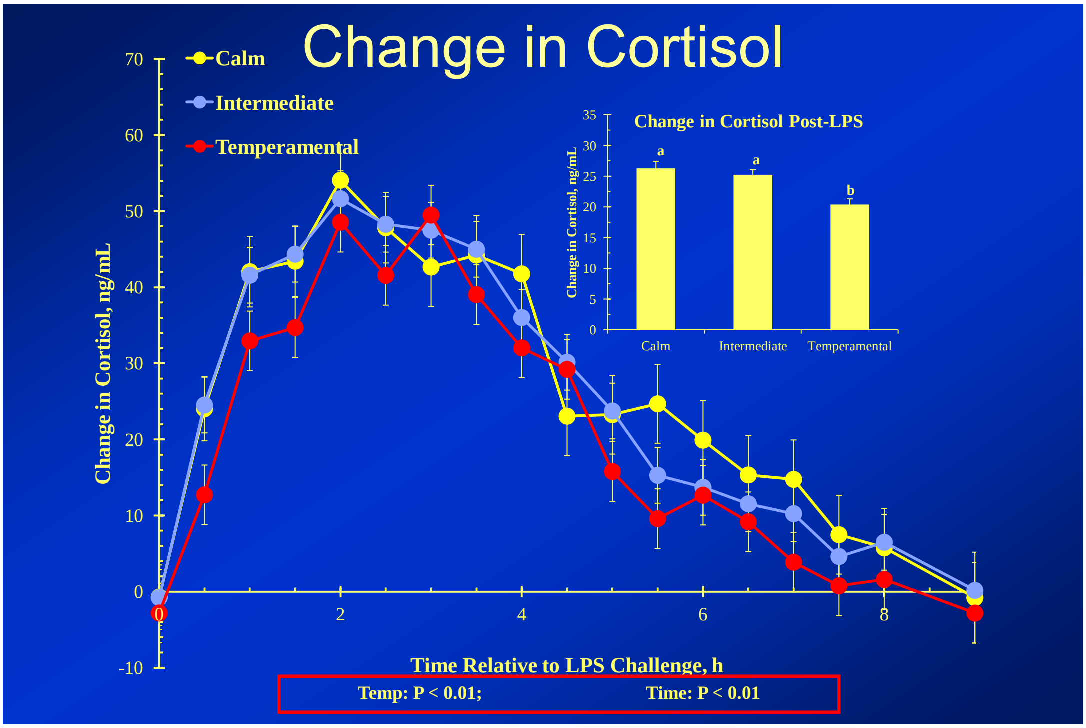
# Rectal Temperature

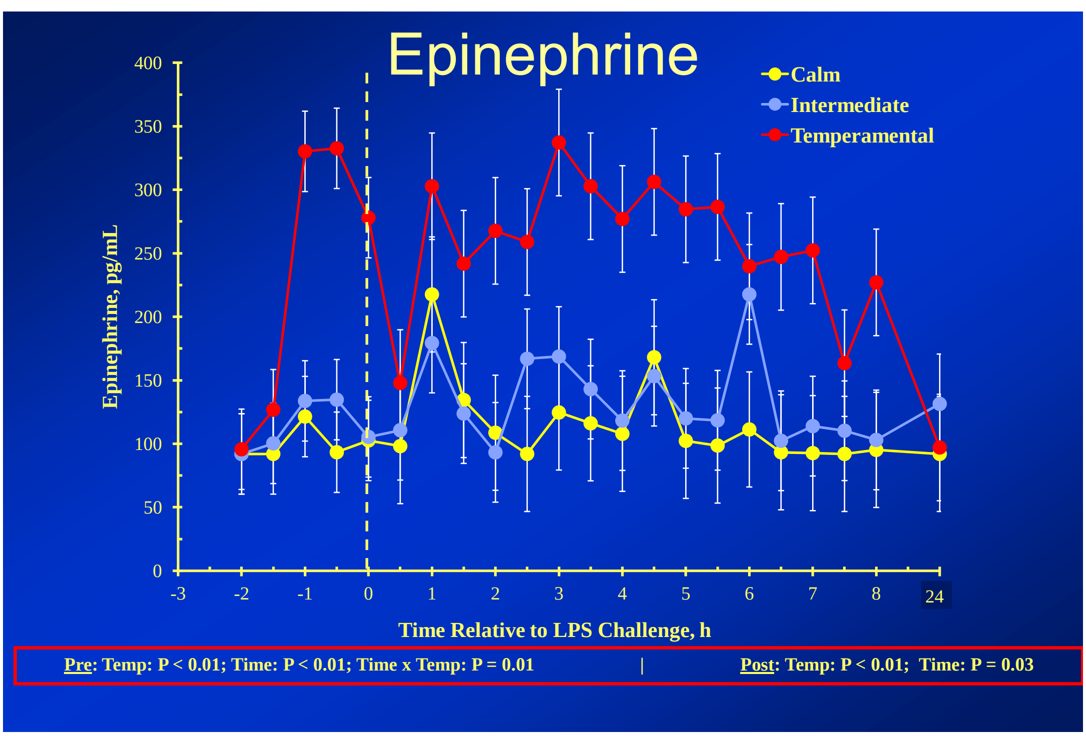



**Pre and Post-LPS: Temp: $P < 0.001$; Time: $P < 0.001$**


Sickness Behavior Scores
Calm
Intermediate
Temperamental
Sickness Behavior Score
† Intermediate bulls differ compared to calm bulls (P < 0.05)
*Temperamental bulls differ compared to calm bulls (P < 0.05)
**Temperamental bulls differ compared to intermediate and calm bulls (P < 0.05)
Time Relative to LPS Challenge, h
Temp: P < 0.001; Time: P < 0.001; Temp x Time: P = 0.064


Cortisol
Calm
Intermediate
Temperamental
Cortisol, ng/mL
Time Relative to LPS Challenge, h
Pre: Temp: P < 0.01; Time: P = 0.07; | Post: Temp: P = 0.80; Time: P < 0.01


Change in Cortisol
Calm
Intermediate
Temperamental
Change in Cortisol, ng/mL
Time Relative to LPS Challenge, h
Temp: P < 0.01;
Time: P < 0.01
Change in Cortisol Post-LPS
Change in Cortisol, ng/mL
Calm
Intermediate
Temperamental
a
a
b


Epinephrine
Calm
Intermediate
Temperamental
Epinephrine, pg/mL
Time Relative to LPS Challenge, h
Pre: Temp: P < 0.01; Time: P < 0.01; Time x Temp: P = 0.01
Post: Temp: P < 0.01; Time: P = 0.03

## Results

1. Temperamental bulls had greater pre-LPS rectal temperature but produced a lesser response to LPS.
2. Temperamental bulls displayed less signs of sickness following LPS administration.
3. Relative to baseline values, temperamental bulls produced a lesser cortisol response to LPS.
4. Temperamental bulls produced greater epinephrine pre- and post-LPS.

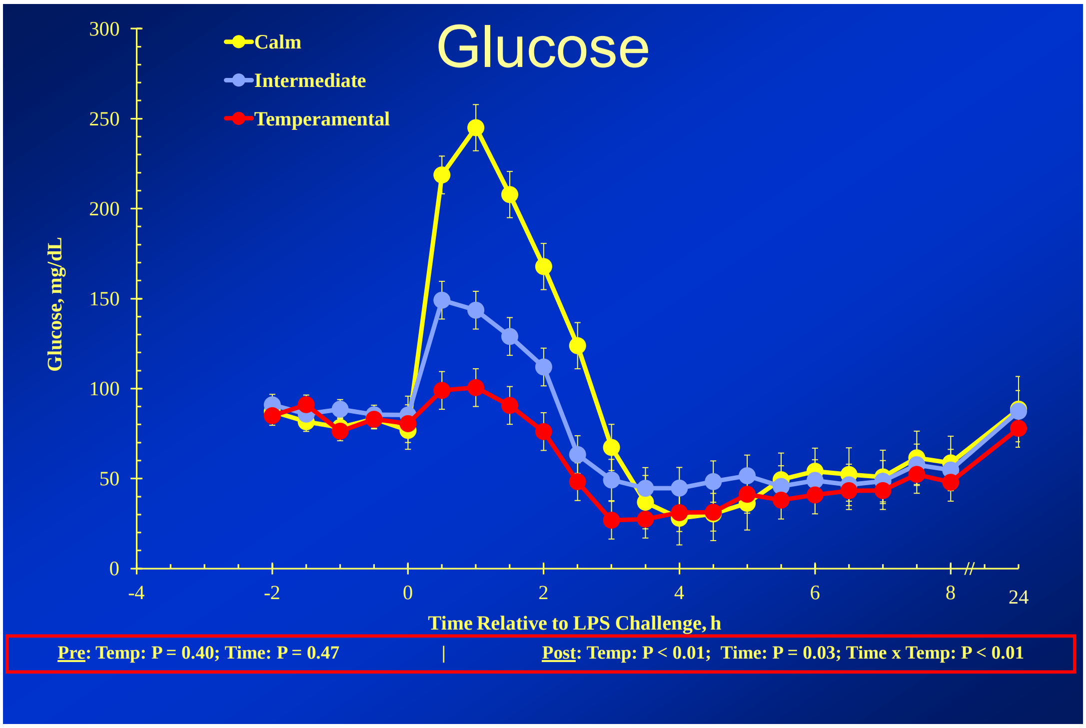
## Conclusions

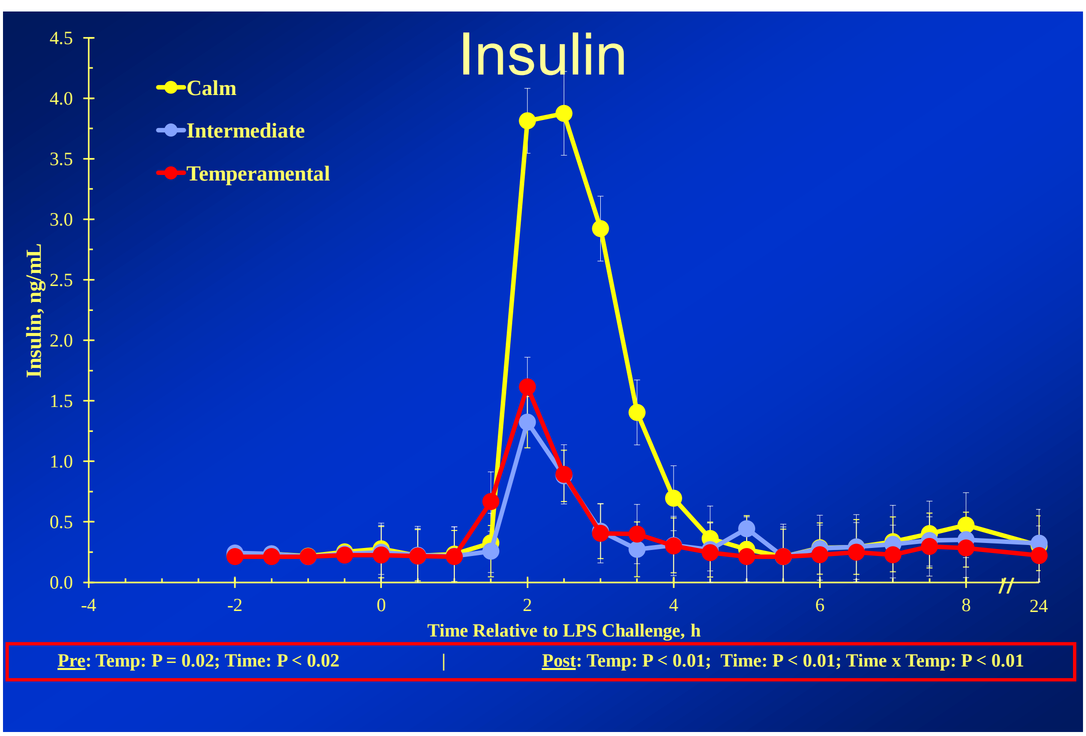
1. Temperament differentially influenced physiological and endocrine responses to LPS challenge.
2. As temperamental cattle do not display as many behavioral signs of sickness, they may increase the risk of infection to calmer cattle.
3. Other factors may be influencing the response of temperamental cattle to LPS.

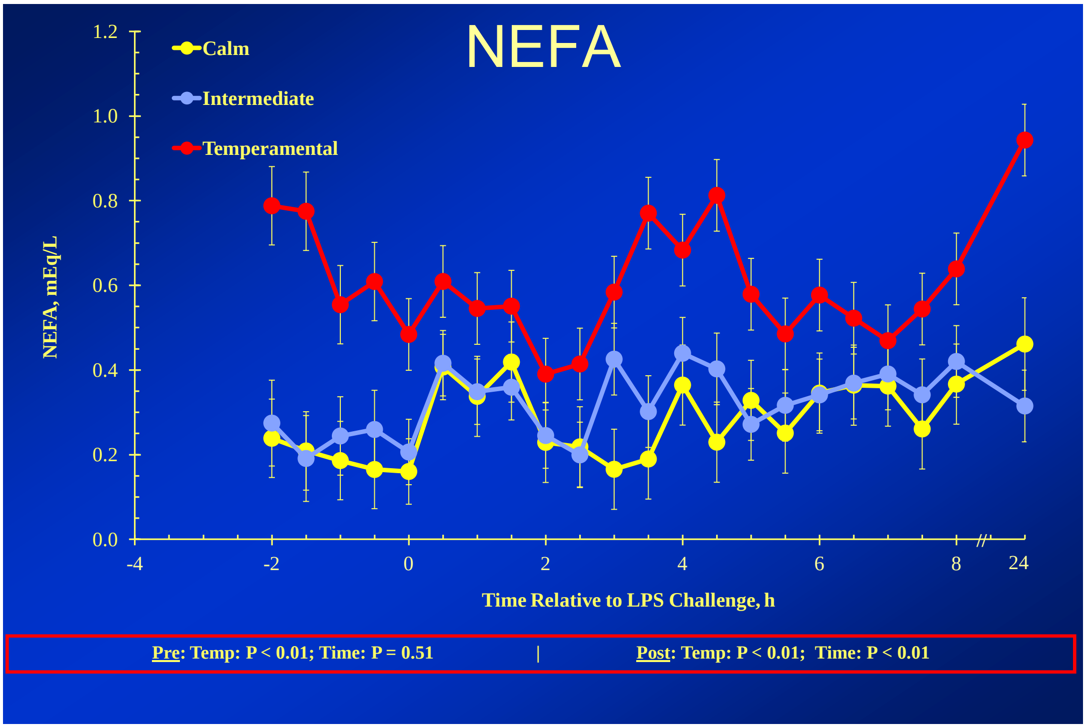
## The immune response has a high energy demand.

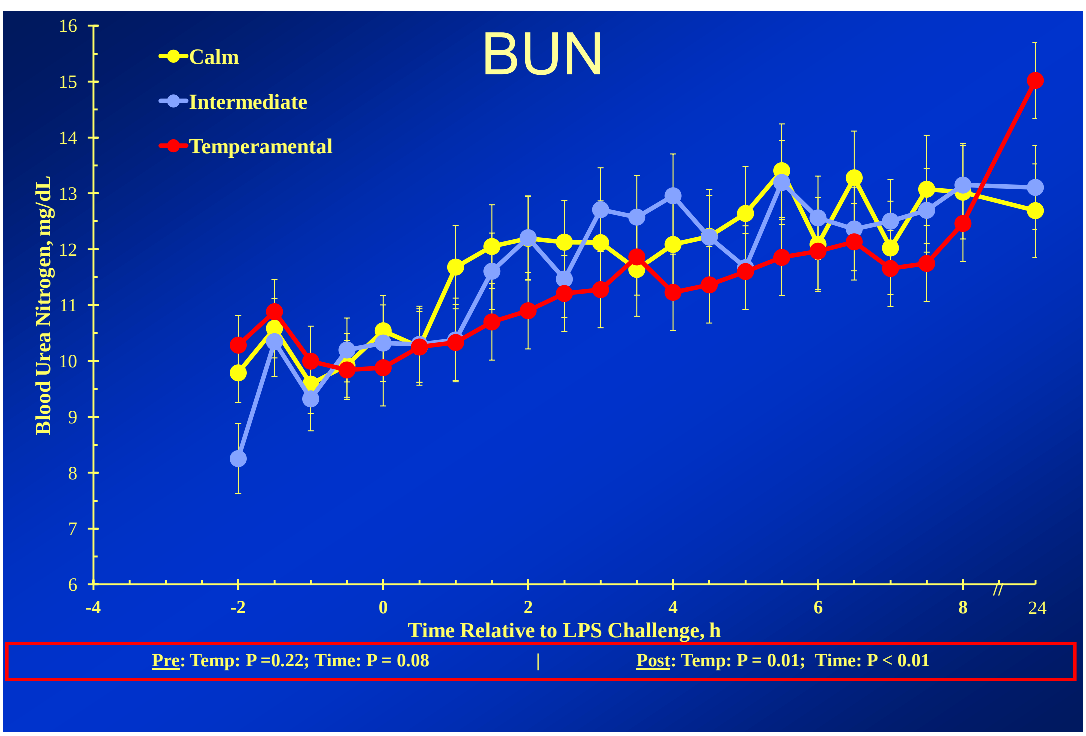
## Hypothesis:

Differences observed in response to an immune challenge are due to differences in energy availability between calm and temperamental cattle.


Glucose
Calm
Intermediate
Temperamental
Glucose, mg/dL
300
250
200
150
100
50
0
-4
-2
0
2
4
6
8
24
Time Relative to LPS Challenge, h
Pre: Temp: P = 0.40; Time: P = 0.47
|
Post: Temp: P < 0.01; Time: P = 0.03; Time x Temp: P < 0.01


Insulin
Calm
Intermediate
Temperamental
Insulin, ng/mL
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-4
-2
0
2
4
6
8
24
Time Relative to LPS Challenge, h
Pre: Temp: P = 0.02; Time: P < 0.02
|
Post: Temp: P < 0.01; Time: P < 0.01; Time x Temp: P < 0.01


NEFA
Calm
Intermediate
Temperamental
NEFA, mEq/L
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-4
-2
0
2
4
6
8
24
Time Relative to LPS Challenge, h
Pre: Temp: P < 0.01; Time: P = 0.51 | Post: Temp: P < 0.01; Time: P < 0.01


BUN
Calm
Intermediate
Temperamental
Blood Urea Nitrogen, mg/dL
16
15
14
13
12
11
10
9
8
7
6
-4
-2
0
2
4
6
8
24
Time Relative to LPS Challenge, h
Pre: Temp: P =0.22; Time: P = 0.08 | Post: Temp: P = 0.01; Time: P < 0.01

## Results

1. Glucose increased in response to LPS in calm and intermediate bulls.
2. Insulin release was greater in calm bulls following LPS.
3. Concentrations of NEFA were greater before and after LPS in temperamental bulls.
4. Concentrations of blood urea nitrogen were lower in temperamental bulls.

## Conclusions

1. Calm bulls became insulin resistant following LPS challenge.
2. Elevated cortisol and epinephrine before LPS challenge of temperamental bulls may reduce subsequent glucose responsiveness.
3. Temperamental bulls use NEFA for energy as they have less glucose available.

Metabolic differences exist between temperaments in cattle.

Separate management strategies should be devised for temperamental cattle to decrease input costs and maximize profit.

# QUESTIONS?