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Evaluation of a Nutritional Calming Supplement for Stress Management in Beef Cattle

Randall Mallette

EVALUATION OF A NUTRITIONAL CALMING SUPPLEMENT FOR STRESS MANAGEMENT IN BEEF CATTLE

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Randall Patrick Mallette
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List of Abbreviations

ACTH	Adrenocorticotropic Hormone
ADG	Average Daily Gain
AI	Artificial Insemination
BAS	Bovine Appeasing Substance
BRD	Bovine Respiratory Disease
BVD	Bovine Viral Diarrhea
BW	Body Weight
CRF	Corticotropin-Releasing Factor
CRH	Corticotropin-Releasing Hormone
DFD	Dark Firm and Dry
DMI	Dry Matter Intake
FE	Feed Efficiency
FTAI	Fixed-Time Artificial Insemination
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
HCW	Hot Carcass Weight
HPA	Hypothalamic-Pituitary-Adrenal
ITS	Individual Temperament Score
LH	Luteinizing Hormone
MES	Modified Exit Score
SAM	Sympathetic-Adrenal-Medullary
TMR	Total Mixed Ration
UV	Ultraviolet

Abstract

Two studies were conducted to determine the efficacy of two formulations of a nutritional calming product, Placid 1.0 and Placid 2.0, at keeping beef cattle in a calm state when exposed to environmental factors that put them into an excited state**.** In the pilot study, nine crossbred beef heifers were orally drenched daily for three days with 60 mL of their respective treatments, pen scores and exit scores were recorded, and blood was collected for cortisol analysis. There were no treatment effects for either treatment on any of the measured behavioral parameters or serum cortisol concentrations.

The products were reformulated after the pilot study to be used in a feeding trial. For this study, thirty Brangus cross steers were fed the products for three days. Pen score, chute score, and exit velocity were measured on d0, 4, and 7. Exit velocities were divided into quintiles and assigned a modified exit score on a scale of 1-5. Pen score, chute score, and modified exit score were averaged to get an individual temperament score (Cooke et al., 2009b). Blood was collected on d0, 4, and 7 for cortisol and macromineral analysis.

Data were analyzed using repeated measures (mixed model) ANOVA in Prism® (GraphPad Prism 9.5.1 for Windows), post-hoc analyses were conducted using Fishers LSD test, and significance was declared at $P<0.05$. There were no treatment effects for any of the measured behavioral parameters or for serum cortisol concentration. There was a main effect of time for chute score and individual temperament score. There was a main effect of time for phosphorus, magnesium, sodium, and chloride. The observed effects of time on behavioral measures of stress can be attributed to acclimation to handling, while the observed differences in

macromineral concentrations are likely due to individual animal variations. All mineral measures fell within the normal ranges for bovines.

Stress levels in beef cattle are an important consideration for the beef industry due to its impact on growth and production, end product, disease susceptibility, and reproduction. Affordable and efficacious calming products could have an important impact on the beef industry by increasing returns in key production areas.

Chapter 1. Introduction

Beef cattle are exposed to many stressful situations throughout their lives. These stressful events include, but are not limited to, handling, weaning, transport, and introduction to novel facilities. A stress response is defined as the reaction to internal and external stimuli affecting the animal's well-being. Temperament, the agitated and/or aggressive behavior of cattle in response to handling, is considered by definition to be a stress response (Cooke, 2009). As a heritable trait, temperament must be considered when discussing stress in cattle due to its direct relationship with the production of stress hormones. Temperament can be detected during the early life of the calf, and this information can be useful to producers as a selection tool (Kasimanickam et al., 2018). Temperament can be measured mechanically using exit velocity (Curley et al., 2014), and is especially effective when combined with more subjective measurements such as chute and pen scores. Stress due to handling practices can significantly impact beef production, and individual animal's reactions to handling stress can vary drastically due to factors such as temperament and previous experiences (Grandin, 1997; Grandin et al., 2015; Waiblinger et al., 2004). These stress reactions influence important economic aspects of beef cattle production including reproduction, growth and production, and end product quality.

Increased stress levels also compromise the immune system, often resulting in increased illness and disease risk. Previously, the use of prophylactic antibiotics in cattle diets was once a common practice to decrease the incidence of illness, as well as promote growth and performance. However, antibiotic resistance concerns have led to the interest in non-pharmacological calming products. The use of non-pharmacological calming products to reduce stress in cattle has garnered interest in recent years. These products do not require a veterinary prescription for use and are readily available.

There are products available that provide calming effects in livestock. The Zinpro ® Placid LQ ® kit is manufactured by Zinpro Specialty Products for calming swine while recovering from stressful situations such as tail biting, transportation, and handling. This product is a zinc amino acid complex, nutritive botanical supplement that is mixed with Aqua X Cell™, a proprietary blend of oregano, thyme, rosemary, and cinnamon. The two products are mixed together and added to the animal's drinking water (Zinpro.com).

Santa Cruz Animal Health also markets a product for cattle, goats, sheep, and pigs called Ultra Cruz®. This product contains calcium, magnesium, and theanine and is recommended for stressed livestock. Santa Cruz claims that this product reduces hyperactivity, encourages relaxation, and promotes focus and calmness (scahealth.com), however there are no published studies on the efficacy of Ultra Cruz®.

With consumer concern for animal welfare becoming more of a deciding factor in how they purchase food, along with the concerns about antibiotic resistance, availability of non-pharmacological calming products has increased. These products are available for swine, sheep, goats, and cattle. As interest in these products continues to increase, manufacturers are developing more options for producers. The objective of this research was to determine the efficacy and animal responses to two formulations of a nutritional calming supplement, Placid 1.0 and Placid 2.0, in beef cattle exposed to environmental factors that put them in an excited state.

Chapter 2. Literature Review

Stress Hormones

Stress can be divided into two categories: acute (short-term) and chronic (long-term). The acute stress response, also known as the "fight or flight" response, is a relatively short-lived stress response regulated by the sympathetic-adrenal-medullary axis (SAM) involving the catecholamines epinephrine and norepinephrine (Sapolsky et al., 2000). During stressful situations, these hormones cause a number of changes in target tissues, including an increase in respiratory rate and heart rate, as well as lipolysis to increase blood glucose levels (Hsieh, 1990). Alternatively, chronic stress is a slower and longer-lived stress response that is regulated by the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky et al., 2000). Cortisol, the stress hormone associated with the HPA axis, is commonly measured as a biomarker of stress because it is present and measurable for a much longer period of time than epinephrine, which has a half-life of only 2–3-minutes. The HPA axis is shown in Figure 1 (Bowden et al., 2019).

After the initial "fight or flight" response, the HPA axis functions to release cortisol in response to stressors, both endogenous and exogenous. When a stressor is recognized, the hypothalamus releases corticotropin-releasing hormone (CRH) into the portal system which is carried to the anterior pituitary where it causes the release of adrenocorticotropic hormone (ACTH). ACTH then acts upon the adrenal gland to cause the release of cortisol. Cortisol has either a positive or negative feedback on the hypothalamus and anterior pituitary, according to circulating levels, to suppress or increase the release of CRH and ACTH (Bowden et al., 2019).

Cortisol has many functions in the body. At normal circulating levels, some functions of cortisol include counteracting insulin to maintain blood glucose, regulating blood pressure, and regulating metabolism (Hsieh, 1990). Under stressful conditions, increased cortisol levels increase blood glucose to continue providing the increased energy level initiated by epinephrine. Increased cortisol levels also have negative feedback on non-essential bodily functions, such as reproductive cyclicity (Dobson, 2001).

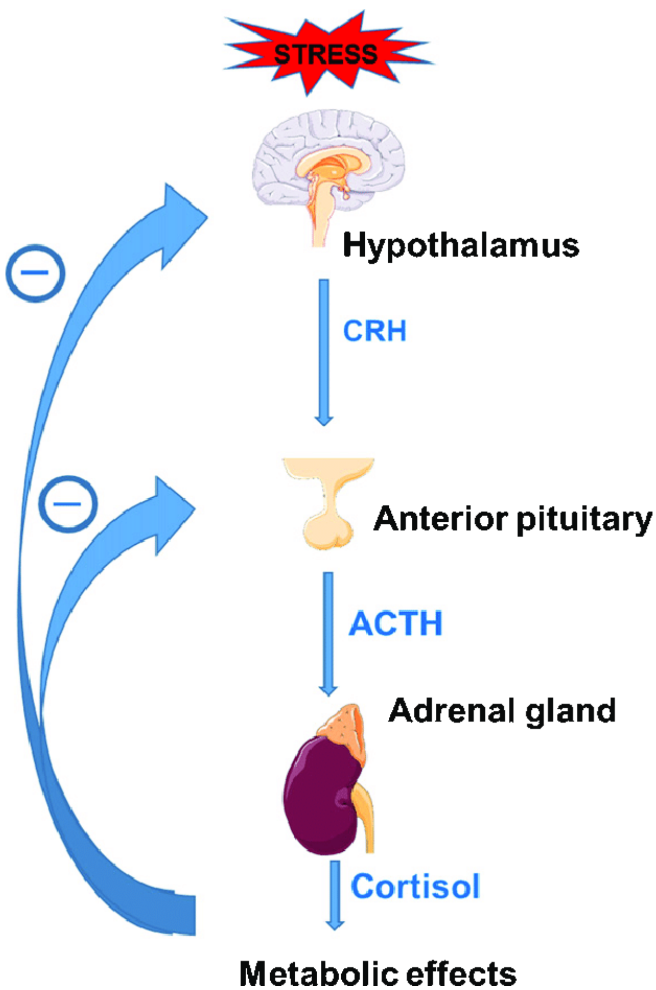

Figure 1. Hypothalamic-pituitary-adrenal axis. Bowden et al., 2019

Growth and Production

Temperamental cattle are especially prone to stress related decreases in production (Café et al., 2011). Studies have shown that transportation stress causes a significant reduction in body weight. Earley et al. (2011) found that weanling heifers transferred from Ireland to France by road and sea lost 7.6% of their body weight, while bulls lost 7% during the same trip. This is likely due to the effect of stress hormones that are produced during transport and handling. Glucocorticoids stimulate secretion of somatostatin by the hypothalamus, inhibiting growth hormone secretion (Kumar et al., 2012), which is responsible for muscle growth and is lipolytic in nature.

Other studies have found that cattle with poor temperaments, as measured by flight speed and chute score, consistently consumed less feed leading to slower growth rates and lower body weights (Café et al., 2011). Voisnet et al. (1997) showed that cattle that become agitated and excited when confined in a squeeze chute have significantly lower weight gains, as well as deleterious end product effects. This study also found that animals with lower temperament scores (1 or 2 on a 1 to 5 scale) had increased average daily gains than those with scores of 3 or higher. Additionally, *Bos taurus* steers with calmer temperaments showed a 0.19 kg/day higher average daily gain compared to steers with higher temperament scores (Voisnet et al., 1997).

Numerous studies have shown that cattle with excitable temperaments exhibit decreased average daily gains (ADG). Cooke (2009) attributes this consistent observation to 3 factors: 1) excitable cattle are more concerned with their surroundings than with intake of forages and feed, 2) the forages and feed that excitable cattle do intake are not efficiently used for gain, due to the animal's altered state, and 3) the physiological changes due to chronic stress, such as increased cortisol levels, negatively impacts their weight gain (Cooke, 2009).

End Product

Pre-slaughter handling stress has been shown to negatively impact the quality of meat. Ensuring that animals are calm prior to slaughter affects the muscle quality on a chemical level, which is responsible for determining the acceptability of meat by consumers. Stress before and during slaughter depletes muscular stores of glycogen, causing a reduced level of lactic acid development in the meat. Lactic acid is responsible for producing tasteful and tender meat, so this low level of lactic acid has considerable consequences on meat quality and shelf life. This condition is referred to as dark firm and dry (DFD) meat. Additionally, lactic acid also limits bacterial growth on the carcass. Depleted levels of lactic acid due to pre-slaughter stress can allow harmful levels of bacterial growth, leading to meat spoilage and possibly consumer sickness. Extreme cases of rough handling or transport can result in severe carcass losses due to excessive bruising, muscle damage, bleeding, and broken bones (Gebregeziabhear et al., 2015).

Pre-slaughter handling also affects meat color and pH which can mean discounted meat values (Lahucky et al., 1998). The unfamiliar environment of a slaughter facility can also have an impact on meat quality. One study exposed cattle to pigs for several hours prior to slaughter, finding that the stress of unfamiliar sounds caused higher meat pH_0 (stressed: 6.73 vs control: 6.53) and pH_{24} (stressed: 5.63 vs control: 5.53) values and increased cooking losses (Pena et al., 2014).

Researchers have suggested slaughter facilities include a feedlot for acclimating animals to the slaughter facility, its sounds, and personnel for a period of time prior to slaughter. This system would also minimize transportation stress just prior to slaughter because the animals would be walked to the slaughter facility from the adjacent feedlot rather than being shipped in

(Njisane et al., 2017). This is a system that could be implemented for slaughter facilities being built in the future but does not address the need for existing slaughter facilities.

Reproduction

The simple economic objective of a cow-calf operation is to wean a calf per cow each year (Fernandez-Novo et al., 2020). The reproductive cycle is dependent upon timely endocrine secretions of reproductive hormones, their recognition in target tissues, and the actions they cause in the target tissues. In the female, secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus causes release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. These hormones act on the ovary, leading to follicle recruitment, selection, dominance, and eventually ovulation. However, during times of stress, the hypothalamic-pituitary-adrenal axis produces hormones that negatively impact GnRH secretion such as corticotropin-releasing factor (CRF), adrenocorticotrophic hormone (ACTH), and glucocorticoids (Kumar et al., 2012). These stress hormones also cause a reduction in the responsiveness of the gonads to sex steroids (Fernandez-Novo et al., 2020). Reducing adrenal steroidogenesis by acclimatizing cattle to humans and handling procedures, which has been shown to hasten onset of puberty (Cooke et al., 2009b; Cooke et al., 2012), is a measure that producers should consider implementing on their operations. Suppression of GnRH secretion through adrenal steroidogenesis can delay important reproductive milestones such as time to puberty or post-partum return to cyclicity.

Pregnancy rates in beef herds can also be negatively affected by handling stress (Cooke et al., 2011) and temperament (Cooke et al., 2009a), and plasma cortisol concentrations in excitable cattle have been shown to be higher than in cattle with a calmer disposition (Cooke et al., 2010; Cooke et al., 2014). As discussed earlier, elevated cortisol levels can impair reproductive events by disrupting GnRH secretion and the post-partum return to cyclicity. However, cortisol also negatively affects the quality of the ovulated oocyte and establishment of pregnancy (Dobson et al., 2001). With these factors in mind, Cooke evaluated the interaction between temperament and pregnancy rates using estrus synchronization and fixed-time artificial insemination (FTAI), finding that pregnancy rates and probability of pregnancy are both decreased in cattle with excitable temperaments (Cooke et al., 2011). It is important for producers who implement reproductive technologies such as estrus synchronization and artificial insemination (AI) to understand the relationship between the stress that animals experience during the extensive handling periods required for these technologies and possible reduction in pregnancy rates.

Non-Pharmacological Products

With the increased interest in the use of non-pharmacological products for reducing stress responses in beef cattle, a number of papers have evaluated some available products. Bovine appeasing substance (BAS) is a synthetic mixture of fatty acids based on the appeasing pheromone. First discovered in swine, appeasing pheromones have been shown to decrease agonistic behavior in piglets (McGlone and Anderson, 2002). The use of appeasing pheromones in cattle has been shown to decrease plasma haptoglobin, a biomarker of stress related inflammation (Cooke et al., 2019). Additionally, Schubach et al. (2020a) found that administration of BAS at weaning alleviated weaning stress. During the first 28 days after weaning, calves in this experiment initially exhibited enhanced ADG and TMR intake and improved behavioral responses, measured using exit velocity and chute score, but there were no improvements in ADG, TMR intake, or FE at the end of the 42-day post-weaning period. Fonesca et al. (2021) found that administration of BAS to *Bos indicus* bulls prior to transport resulted in improved ADG (+ 0.156 kg/d), DMI (+ 0.36 kg/d), and FE (+ 10g/kg), leading to higher HCW (+ 8.8 kg) and dressing percentage (+ 0.15%) compared to the control. Fonesca et al. (2021) also found that BAS administration after feedlot entry only improved performance during the adaptation period. In contrast, Columbo et al. (2020) found that steers receiving BAS after transport did have increased ADG, but this increased ADG was due to increased FE, as feed intake was similar among treatments (2020).

Stress induced disease, especially bovine respiratory diseases (BRD), is a significant problem among stressed animals, with up to 60% of animals being affected in feedlots (Snowder et al., 2006). Columbo et al. (2020) exposed steers to the stress of weaning, auction, transport, commingling, vaccination, and feedlot entry in a 72-hour period prior to administration of BAS at feedlot entry. This study found that while BAS appeared to increase occurrence of BRD, the symptoms were detected earlier than in the control group and required fewer treatments for recovery. When administered at weaning, calves that received BAS had increased serum concentrations of antibodies against BVD (calves were vaccinated on d-21 relative to weaning) (Shubach et al., 2020b). A French study administered BAS to young bulls at the beginning of the fattening period to assess its effect on BRD incidence. This study found that on day 8, there were more clinical signs of respiratory disease observed in the BAS group compared to the control group (26 vs 10, respectively). However, on day 30, there were fewer clinical signs of respiratory disease observed in the BAS group compared to the control group (6 vs 13, respectively) (Hervet et al., 2021).

Another non-pharmacological approach to improvement of immune system function and production parameters was evaluated in Italy using a nutraceutical mixture based on live yeast, mannan oligosaccharides, and organic selenium. Cattle were supplemented with this mixture for 30 days and evaluated for BRD and other health issues as well as growth performance and carcass characteristics after a fattening period lasting 187 days. This study found that the nutraceutical mixture improved immune system defenses, significantly reducing the morbidity of BRD compared to the control (51.2% vs 60.2%, respectively) as well as fewer relapsed cases (9.6% vs 16.3%, respectively). Immune system reactivity was improved by the treatment, and treated animals weighed significantly more than the control animals at the end of the fattening period (519.01 +/- 32.4 kg vs 514.81 +/- 24.81 kg, respectively). No differences were reported for carcass characteristics (Grossi et al., 2021).

Mineral supplementation is an important component of beef cattle production, playing numerous important roles in bodily processes. Deficiencies of copper and iron have both been shown to depress the function of the immune system, increasing the risk of infections (Scaletti, 2003; Mollerberg and Moreno-Lopez, 1975). Suttle demonstrated the importance of copper in sheep, finding that the immune system consumes copper when fighting infections, therefore increasing copper requirements (Suttle, 2012). Ceruloplasmin, an acute-phase protein associated with stress reactions, is also modulated by copper intake. Iron levels are rarely low due to iron intake from soil while foraging, however increased morbidity and mortality from depressed immune responses has been documented (Mollerberg and Moreno-Lopez, 1975).

Chapter 3. Materials and Methods

Two projects were conducted to determine if two formulations of a nutritional calming product, Placid 1.0 and Placid 2.0 (Zinpro Corporation), will keep beef cattle in a calm state when exposed to environmental factors that put them into an excited state. The first study was a pilot project to determine if either product would improve temperament and decrease excitability when handled. Study 2 was a feeding trial to further assess the products after reformulation based on results of the pilot project. All procedures for these experiments were approved by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee.

Study 1: Pilot Study

Nine crossbred beef heifers (mean BW = 374.35 kg) at the LSU AgCenter Doyle Chambers Central Station research farm in Baton Rouge, Louisiana, were used in the study conducted in October 2022. Heifers were randomly assigned to one of three treatments, n = 3 per treatment, which included water (control), Placid 1.0, and Placid 2.0 (products manufactured by Zinpro Corp.). Prior to initial treatment, pen scores were assigned to each animal. Pen scores were assessed using the scoring scale as described by Cooke (2010). After pen scores were assigned, animals were moved to the squeeze chute and body weights recorded. Blood samples were collected via jugular venipuncture into 6 mL evacuated tubes with no anticoagulant for analysis of cortisol. After the blood sample was collected, each animal was administered 60 ml of its respective treatment via oral drench. Exit speed scores were assigned as animals left the chute using the scoring system of 1 = walk, 2 = trot, 3 = canter, and 4 = run (Lanier and Grandin, 2002). Animals were kept in one pasture for the duration of the 4-day project and were given their treatment daily for 3 days. On d4, pen scores and exit scores were measured, and blood samples were collected as described previously.

Cortisol Analysis

Upon completion of blood sample collection on d0 and d4, samples were centrifuged for 15 minutes at 600 x g, serum was collected, protected from UV light, and stored at -20°C until analysis. Serum cortisol concentrations were determined using radioimmunoassay kits (MP Biomedical Cortisol Coated Tube RIA Kit, Irvine, CA).

Study 2: Feeding Trial

Animals and Dietary Treatments

A feeding trial was conducted at the LSU AgCenter Hill Farm Research Station in Homer, Louisiana in January 2023. On d-1, 66 Brangus-cross steers, all born between February and April of 2022, were weighed and sorted into pens according to body weight (<249.48 kg., s249.48 kg. to 294.84 kg., >294.84 kg. All nineteen steers in the 249.48 kg. to 294.84 kg. group were selected for use in the study, as well as four from the <249.48 kg. group and seven from the >294.84 kg. group. Selected steers (n=30, mean BW = 268.07 kg) were ranked according to body weight, then randomly assigned to one of 3 treatments consisting of control (diet only with no supplemental product; n = 10), treatment 1 (Placid 1.0; n =10), or treatment 2 (Placid 2.0; n = 10). Animals were then sorted into their respective study pastures with 5 steers per pasture. Pastures were identical in size (1.214 hectares), with free access to water and ad libitum hay (see Table 3 for hay analysis). Pastures 1 and 2 were control groups, pastures 3 and 4 received treatment 1, and pastures 5 and 6 received treatment 2. The steers remained in their respective pastures for 3 days. During this time, all steers had free access to water and were fed a ration at

2% of the mean BW for each pasture. Ration and treatment amounts per day are shown in Table 4. Treatments 1 and 2 (pastures 3-6) were top dressed over the ration at a rate of 0.454 kg of product per 317.5 kg of body weight. From d4 until the end of the study, steers received the same ration but with no treatment added.

Data and Sample Collection

On d0, 4, and 7, steers were moved from their assigned pasture to pens for assessment of pen scores. Pen score is a non-restrained technique that evaluates the behavioral responses observed with a single evaluator standing inside the pen. Once inside the pen, the evaluator moves 3 steps directly toward the animal(s) and observes the response on a scale of 1 to 5 where 1 = unalarmed and unexcited, walks slowly away from the evaluator, 2 = slightly alarmed, trots away from the evaluator, 3 = moderately alarmed and excited, runs away from the evaluator, 4 = very alarmed and excited, runs with head held high and may charge the evaluator, or 5 = very excited and aggressive in a manner that requires evasive actions by the evaluator to avoid contact (Cooke, 2010).

After pen score assessment, animals were walked 0.805 km to a working facility for further data and sample collection. Body weight was recorded, and then the animal entered the squeeze chute for score assessment. Chute score assessment is a technique in which cattle are individually restrained in the chute and scored on a 1 to 5 scale according to their behavior. Scoring guidelines include 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, shaking of the chute, and 5 = violent and continuous struggling (Cooke, 2010). After chute scores were assigned, blood samples were collected via coccygeal venipuncture into 6 mL evacuated tubes with no anticoagulant for analysis of cortisol, calcium, chloride, phosphorus, magnesium, sodium, and potassium.

Table 1. Definition of pen score scoring system

1	Unalarmed and unexcited, walks slowly away from the evaluator
2	Slightly alarmed, trots away from the evaluator
3	Moderately alarmed and excited, runs away from the evaluator
4	Very alarmed and excited, runs with head held high and may charge the evaluator
5	Very excited and aggressive in a manner that requires evasive actions by the evaluator to avoid contact

Table 2. Definition of chute score scoring system

1	Calm with no movement
2	Restless movements
3	Frequent movement with vocalization
4	Constant movement, vocalization, shaking of the chute
5	Violent and continuous struggling

Once blood samples were collected, animals were released from the squeeze chute and exit velocity was recorded using a timer trip system. The distance from the head catch to the first timer was 2.75 meters, and the distance between trip timer 1 and 2 was 2.0 meters.

Exit velocity is a non-restrained technique that evaluates the speed of an individual animal immediately after it leaves the squeeze chute. As the speed increases, the more frightened the evaluated animal may have been due to the human handling in the chute. Exit velocity can be evaluated in actual speed measures (i.e., meters/second) or on visual estimates. To determine actual speed, the evaluator needs to establish a known distance, or route, that the animal will travel after leaving the chute (measured in meters), and then calculate the time required for the animal to travel the route (in seconds). The evaluator can use a chronometer or infrared electronic timers, such as those used in rodeo events. How the route is established is an important consideration. If it begins too close to the chute, temperamental cattle can slip when exiting the chute and therefore need more time to travel through the route. Also, the route should not be too lengthy and/or established too far away from the chute, otherwise calm cattle may stall, whereas temperamental cattle can calm down and decelerate in the middle of the route.

Once all data and samples were collected, animals were then sorted back into their respective groups in pens at the working facility. Animals were then returned to their study pastures via trailer.

Cortisol and Mineral Analysis

Upon completion of blood sample collection on d0, 4, and 7, samples were centrifuged for 15 minutes at 600 x g, serum was collected, protected from UV light, and stored at -20°C until analysis. Serum cortisol concentrations were determined using radioimmunoassay kits (MP Biomedical Cortisol Coated Tube RIA Kit, Irvine, CA). Serum was analyzed for macromineral concentrations at the Michigan State University Veterinary Diagnostic Laboratory (Lansing, MI).

Statistical Analysis

In the feeding trial, exit velocity data for each day was divided into quintiles, then assigned a modified exit score (MES) on a scale of 1 to 5 with the fastest exit velocities (shortest measured times) receiving a MES of 5 and the slowest exit velocities (longest measured times) receiving a MES of 1. After assigning modified exit scores, each steer was given an individual temperament score (ITS) for each measurement day by averaging the pen score, chute score, and MES (Cooke et al., 2009b).

Data were analyzed using repeated measures (mixed model) ANOVA in Prism® (GraphPad Prism 9.5.1 for Windows), post-hoc analyses were conducted using Fishers LSD test, and significance was declared at $P<0.05$. Pilot study response variables included pen score, modified exit score, and cortisol, while pen score, chute score, MES, ITS, cortisol, Mg, Ca, P, Na, K, and Cl were measured during the feeding trial. Fixed effects included treatment, day, and treatment by day interaction, and random effects were animal within treatment and pen within treatment for the pilot study and feeding trial, respectively.

Table 3. Chemical analysis of hay

Nutrient	**As received**	**Dry matter basis**
Dry matter content	86.74	100.00
CP (Crude protein)	4.90	5.65
ADF (acid detergent fiber)	36.81	42.44
NDF (Neutral Detergent Fiber)	64.03	73.82
Ash	2.35	2.71
Fat	1.90	2.19
Lignin	4.30	4.95
Ca	0.28	0.32
P	0.13	0.15
K	0.99	1.14
Mg	0.13	0.15
ESC (Ethanol Soluble Carbohydrate = Simple sugars)	1.67	1.92
WSC (Water Soluble Carbohydrate)	5.10	5.88
TDN (Total Digestible Nutrient)	47.89	55.22

DMI (Dry Matter Intake), % Live body weight: 1.99
RFQ (Relative Forage Quality): 89

Table 4. Amounts of ration and treatment offered during days 1 through 3.

Pasture	Ration/day (kg)	Treatment	Treatment/day (kg)
1	26.3	Control	0
2	26.7	Control	0
3	26.4	1	1.90
4	27.3	1	1.95
5	26.6	2	1.90
6	27.5	2	1.95

Chapter 4. Results and Discussion

Pilot Study

Least squares means for pen score in heifers administered nutritional calming products are reported in Table 5. Least squares means for modified exit score in heifers administered nutritional calming products are reported in Table 6. There were no treatment effects (P>0.05) on pen score or modified exit score. The data indicates that administering 60 mL of these nutritional calming products via oral drench had no effect on behavioral measurements of stress.

Least squares means for serum cortisol concentrations in heifers administered nutritional calming products are reported in Table 7. There were no treatment effects (P>0.05) on serum cortisol concentrations. The data indicate that administering 60 mL of these nutritional calming products via oral drench had no effect on serum cortisol levels.

Feeding Trial

Least squares means for pen score, chute score, exit velocity, modified exit score, and individual temperament score for steers fed nutritional calming products are reported in Tables 8, 9, 10, 11, and 12, respectively. There were no treatment effects in steers fed nutritional calming supplements for pen score (P>0.05), chute score (P>0.05), exit velocity (P>0.05), modified exit score (P>0.05), or individual temperament score (P>0.05), however there was a main effect of time for chute score (P<.01) and individual temperament score (P<0.05). The data indicate that feeding the nutritional calming products had no effect on behavioral measurements of stress.

Least squares means for serum cortisol concentrations for steers fed nutritional calming products are reported in Table 13. There were no treatment effects for cortisol in steers fed nutritional calming products (P>0.05). The data indicate that feeding the nutritional calming products had no effect on serum cortisol levels.

The observed effect of time on chute score is supported by acclimation studies. Cooke et al. found that when heifers were exposed to an acclimation process three times a week, chute scores were decreased (Cooke et al., 2009b). In the present study, the heifers were handled daily for four days, and the steers were handled four times in seven days. Heifers were weighed and scored on d0, then drenched daily for three days while the steers were handled for sorting on d-1, then for scoring and blood collection on d0, 4, and 7. However, the observed effect of time on individual temperament score in the present study is not supported by the above study.

The same study above noted that cortisol concentrations were decreased after acclimatization to handling three times a week (Cooke et al., 2009b), however the present study does not corroborate those results. There was a numerical decrease in cortisol levels from d0 to d7, however it was not statistically significant. It should be noted that the animals used in this study are a research herd that, to a degree, are somewhat accustomed to some human interaction, although not as intense or as often as what they experienced in the present study. Cortisol concentrations are used as an indirect measure of stress in animals, including cattle. Latif et al. (2017) measured serum cortisol concentrations to assess stress levels in beef cattle at slaughter. These researchers investigated four methods of animal treatment prior to slaughter, including stunning by percussive captive bolt, stunning by pneumatic captive bolt, no stunning with use of a restraining box, and a conventional method of no stunning with use of a rope to forcefully throw down the cattle. Mean serum cortisol concentrations were 2.24, 1.93, 2.49, and 4.49 ug/ml, respectively, for the four handling methods. These cortisol values are similar to those observed in the current study, indicating that the values are similar to those observed in other stressed beef cattle.

Least squares means for serum macromineral concentrations in steers fed nutritional calming products are reported in Table 12. There were no treatment effects for calcium ($P>0.05$), phosphorus ($P>0.05$), magnesium ($P>0.05$), potassium ($P>0.05$), sodium ($P>0.05$), or chloride ($P>0.05$) in steers fed nutritional calming products. A main effect of time was detected for phosphorus ($P<0.05$), magnesium ($P<0.01$), sodium ($P<0.05$), and chloride ($P<0.01$) in steers fed nutritional calming products. There were numerical increases observed for serum magnesium and serum chloride concentrations, as well as a numerical decrease in serum sodium concentrations, during the study. However, all mineral measures, regardless of statistical significance, fall within the normal bovine levels, therefore there is no biological significance (eClinpath, 2023).

The ingredients in Placid 1.0 and Placid 2.0 are proprietary information, however, calming products are often a mixture of minerals, amino acids, herbs, and/or botanicals. A number of calming products for livestock species include magnesium, which has been shown to influence relaxation of muscles and the nervous system in horses (Dodd et al., 2015), and a deficiency is associated with anxiety (Sartori, 2012). In pigs, there have been mixed results regarding magnesium and stress, but magnesium has been shown to decrease stress associated with transportation when simulated in a vibration crate. However, the same study also found that administering magnesium through the pigs' water for two days prior to transport returned to baseline cortisol slower than control pigs (Peeters, 2005). Other studies found that magnesium supplementation results in decreased skin lesions, an indicator of aggressive behavior (O'Driscoll et al., 2013a; O'Driscoll et al., 2013b). The method of administration and timing of magnesium supplementation in relation to occurrence of a stressor seems to be an important consideration.

L-theanine is an amino acid derivative that shows promise in reducing stress, though no studies have been conducted on livestock species, but can be found in some equine calming products. In humans and mice, L-theanine has been shown to reduce stress responses (Kimura et al., 2007; Wakabayashi et al., 2012). Tryptophan, an essential amino acid, is commonly associated with sleepiness after a large Thanksgiving meal and can also be found in calming products. Though it does appear to decrease cortisol levels in horses, results are unclear (Davis et al., 2017).

Table 5. Least squares means for pen score in heifers administered nutritional calming products[1]

Day	Control	Treatments Placid 1.0	Placid 2.0	SEM[2] C vs Trt 1	C vs Trt 2
0	3.000	4.000	4.000	0.500	0.500
4	2.667	2.000	1.667	0.401	0.333

[1] Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 6. Least squares means for exit score in heifers administered nutritional calming products[1]

Day	Control	Treatments Placid 1.0	Placid 2.0	SEM[2] C vs Trt 1	C vs Trt 2
0	2.000	3.000	1.333	0.428	0.333
4	2.000	3.000	1.667	0.500	0.477

[1] Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 7. Least squares means for serum cortisol concentrations (µg/ml) in heifers administered nutritional calming products[1]

		Treatments		SEM[2]	
Day	Control	Placid 1.0	Placid 2.0	C vs Trt 1	C vs Trt 2
0	2.507	2.743	3.393	0.332	0.601
4	2.290	2.177	1.737	0.427	0.404

[1] Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 8. Least squares means for pen score in steers fed nutritional calming products[1]

		Treatments		SEM[2]	
Day	Control	Placid 1.0	Placid 2.0	C vs Trt 1	C vs Trt 2
0	3.100	3.000	3.000	0.050	0.050
4	3.100	3.000	2.500	0.050	0.271
7	3.000	3.000	2.500	0.000	0.250

[1]Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 9. Least squares means for chute score in steers fed nutritional calming products[1]

		Treatments		SEM[2]	
Day	Control	Placid 1.0	Placid 2.0	C vs Trt 1	C vs Trt 2
0	3.900	3.200	4.000	0.320	0.171
4	2.700	2.500	2.500	0.082	0.141
7	3.000	2.500	2.500	0.189	0.150

Main effect of time ($P<.01$)

Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 10. Least squares means for exit velocity in steers fed nutritional calming products[1]

Day	Control	Treatments Placid 1.0	Placid 2.0	SEM[2] C vs Trt 1	C vs Trt 2
0	0.844	0.959	0.769	0.102	0.059
4	0.894	1.064	0.757	0.098	0.106
7	0.893	1.067	0.935	0.074	0.052

[1] Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 11. Least squares means for modified exit score in steers fed nutritional calming products[1]

Day	Control	Treatments Placid 1.0	Placid 2.0	SEM[2] C vs Trt 1	C vs Trt 2
0	2.900	2.800	3.300	0.427	0.480
4	3.200	2.100	3.700	0.457	0.499
7	3.400	2.400	3.200	0.311	0.191

[1]Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 12. Least squares means for individual temperament score in steers fed nutritional calming products[1]

Day	Control	Treatments Placid 1.0	Placid 2.0	SEM[2] C vs Trt 1	C vs Trt 2
0	3.300	3.000	3.430	0.238	0.212
4	3.000	2.535	2.900	0.175	0.261
7	3.135	2.635	2.735	0.162	0.161

Main effect of time ($P<.05$)

[1]Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 13. Least squares means for serum cortisol concentrations (μg/ml) in steers fed nutritional calming products[1]

Day	Control	Treatments Placid 1.0	Placid 2.0	SEM[2] C vs Trt 1	C vs Trt 2
0	2.145	2.157	1.513	0.146	0.249
4	1.761	2.075	1.796	0.166	0.140
7	1.103	1.894	1.358	0.229	0.079

[1] Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.

[2]SEM = Standard Error of the Mean

Table 14. Least squares means for serum macromineral concentrations in steers fed nutritional calming products[1]

	Treatments			SEM[2]	
Item	Control	Placid 1.0	Placid 2.0	C vs Trt 1	C vs Trt 2
Calcium, mg/dl					
Day 0	9.32	9.29	9.28	0.039	0.060
Day 4	9.19	9.05	9.54	0.079	0.082
Day 7	9.13	9.10	9.18	0.045	0.043
Phosphorus, mg/dl[3]					
Day 0	7.59	7.40	8.07	0.144	0.158
Day 4	6.96	7.48	6.45	0.273	0.213
Day 7	7.50	7.05	6.93	0.214	0.298
Magnesium, mg/dl[4]					
Day 0	2.16	2.16	2.19	0.037	0.036
Day 4	2.49	2.50	2.49	0.062	0.057
Day 7	2.64	2.51	2.57	0.061	0.033
Potassium, mmol/L					
Day 0	5.18	5.26	5.61	0.101	0.161
Day 4	5.18	5.15	5.41	0.095	0.131
Day 7	5.09	4.70	5.17	0.121	0.134
Sodium, mmol/L[5]					
Day 0	143.7	143.3	144.2	0.208	0.838
Day 4	144.7	142.9	141.5	0.766	1.103
Day 7	140.8	140.7	142.0	0.096	0.606
Chloride, mmol/L[6]					
Day 0	98.0	98.9	98.2	0.330	0.455
Day 4	99.8	101.6	100.9	0.597	0.403
Day 7	98.6	98.8	100.2	0.191	0.589

[1] Placid 1.0 and Placid 2.0 are nutritional calming products manufactured by Zinpro, Corp.
[2]SEM = Standard Error of the Mean.
[3]There was a main effect of time (P <0.05).
[4]There was a main effect of time (P <0.01).
[5]There was a main effect of time (P < 0.05).
[6]There was a main effect of time (P< 0.01).

Chapter 5. Summary and Conclusion

Summary

Nine crossbred beef heifers were utilized in a pilot study to determine if administration of 60 mL of two formulations of a nutritional calming product, Placid 1.0 and Placid 2.0, via oral drench were effective in reducing cortisol and behavioral parameters of stress. Heifers were drenched with the products daily for 3 days. Heifers were kept on pasture for the duration of the pilot study.

The products had no effect on pen score, exit score, or cortisol in the pilot study, so the product was reformulated for the feeding trial. For the feeding trial, 30 Brangus-cross steers were utilized in a feeding study using the same products from the pilot study. Steers were randomly assigned to receive the control, Placid 1.0, or Placid 2.0. Treatments were top dressed on the steers' ration. Steers were fed the products for 3 days. Pen score, chute score, and exit velocity were measured on d0, 4, and 7, and using these measurements, modified exit scores and individual temperament scores were also assigned. Cortisol levels were also measured on these days. There were no treatment effects found for either product. However, there were main effects of time for individual temperament score and chute score. Macrominerals were also measured. There were main effects of time for phosphorus, magnesium, sodium, and chloride. However, all mineral levels were within the normal concentration levels for bovines, therefore there is no biological significance.

Conclusion

The data suggest that at the dosages used (60 mL via drench for the pilot study and 0.454 kg of product per 317.5 kg of body weight in the feeding study), Placid 1.0 and Placid 2.0 were not effective in reducing the serum cortisol levels or behavioral measures of stress in beef cattle. Additional research with these products is warranted with a higher number of observations, further reformulation, dosage adjustments, and/or longer feeding period.

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Vita

Randall Mallette was born in Mobile, Alabama to Patrick and Romona Mallette. Randall graduated from George County High School in Lucedale, Mississippi in 2007 and began studies at Mississippi State University. In 2012, Randall received his Bachelor of Science degree in Animal and Dairy Sciences from Mississippi State University. Randall began graduate studies in the spring of 2018 at Louisiana State University Shreveport, then transferred to Louisiana State University Baton Rouge in 2019. Randall anticipates graduating in the summer of 2023, with the degree of Master of Science in Animal Sciences. Randall is currently employed by LSU AgCenter as county agent in Natchitoches and Red River parishes and will continue in that capacity upon graduation.