EFFECTS OF THE BOVINE SLICK MUTATION ON HEAT STRESS RESPONSES AND HAIR GROWTH IN MICE

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SHELBI DANIELLE PERRY

Dr. Timothy J. Safranski, Thesis Advisor

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

EFFECTS OF THE BOVINE SLICK MUTATION ON HEAT STRESS RESPONSES AND HAIR GROWTH IN MICE

presented by Shelbi Danielle Perry,

a candidate for the degree of Master of Science in Animal Science, and hereby certify that, in their opinion, it is worthy of acceptance.

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Dr. Timothy Safranski

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Dr. Kevin Wells

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Dr. Matthew Lucy

_____

Dr. Scott Poock

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EFFECTS OF THE BOVINE SLICK MUTATION ON HEAT STRESS RESPONSES AND HAIR GROWTH IN MICE

Shelbi D. Perry

Dr. Timothy J. Safranski, Thesis Advisor

ABSTRACT

The bovine slick mutations result from more than one allele variant causing a slick hair phenotype and improved thermotolerance to elevated ambient temperatures in cattle. These mutations result in the truncation of the prolactin receptor (PRLR) by 85-120 amino acids. The objective of this project was to test whether genetically modified mice with a similar truncated PRLR showed improved thermal tolerance and/or a hair phenotype. Mice were housed in environmental chambers that increased at 3^{o}C increments every other day from 22^{o}C until 34^{o}C was reached. During this time feed disappearance (FD), water disappearance (WD), tail temperature (TT), and nest scores (NS) were recorded daily. Due to the association between water disappearance and feed disappearance in rodents fed pelleted diets, water disappearance per unit feed disappearance (W/F) was calculated. Female mice had a higher FD at 34^{o}C, WD at 31°C and 34°C, and W/F ratio at 28°C, 31°C, and 34°C ($P<0.05$), but no differences were observed at lower temperatures ($P>0.05$). Genotype did not affect FD at 28^{o}C or 31^{o}C or W/F at 28^{o}C, 31^{o}C or 34^{o}C ($P>0.05$). For W/F, mice heterozygous for the mutation (WT/MUT) and homozygous mutant (MUT/MUT) mice had higher ratios than homozygous wild type (WT/WT) at 22^{o}C and 25^{o}C ($P<0.05$). At 34^{o}C there was a tendency for WT/WT to have a higher ratio than MUT/MUT. FD shows a similar trend

with no differences at 28°C or 31°C. At 34°C WT/WT (2.43 ± 0.03) yielded a significantly lower FD compared to WT/MUT (2.55 ± 0.03) and MUT/MUT (2.60 ± 0.05) ($P<0.05$). At 22°C WT/WT mice (4.41 ± 0.08) consumed a higher FD than WT/MUT (4.19 ± 0.06) and MUT/MUT (4.13 ± 0.12) ($P<0.05$). Males and females showed different TT (F= 28.84 ± 0.0.05 vs. M= 29.07 ± 0.06; $P<0.05$). WT/MUT mice had warmer TT than MUT/MUT mice (WT/MUT= 29.07 ± 0.04 vs. MUT/MUT= 28.81 ± 0.08; $P<0.05$). However, WT/MUT versus WT/WT did not differ ($P>0.05$). No differences existed among genotypes at each temperature for NS in females ($P>0.05$). Male NS did not differ at 34°C based on genotype; however, WT/MUT and MUT/MUT had higher NS than WT/WT at lower temperatures. At three weeks of age, a 1.5 cm x 1.5 cm patch was shaved on the back over the hip region on each mouse. Visual appraisals of hair regrowth were monitored daily. No difference due to genotype was observed ($P<0.05$); however, sex had a drastic effect on days to hair regrowth with males taking less time to show regrowth ($P<0.05$). In conclusion, the bovine slick mutation did appear to improve heat stress responses in mice and introduced a novel phenotype during periods of cold stress; however, since no hair phenotype was observed these effects must be acting through another mechanism, not simply due to variation in hair.

CHAPTER 1:

INTRODUCTION

Heat stress caused by increased ambient temperatures negatively affects many livestock industries. The total economic loss has been estimated to be $1.7 billion US dollars across all livestock industries when heat abatement strategies are utilized. The swine industry constitutes $299 million of the total, beef and dairy industries contribute $1.26 billion, and poultry adds $128 million (St-Pierre et al., 2003). Due to this, breeds with superior heat thermotolerance have been the target of recent research to determine what causes this thermoregulatory advantage (Littlejohn et al., 2014; Porto-Neto et al., 2018).

In Senepol and Limonero cattle, the bovine slick mutations have been repeatedly shown to improve thermotolerance to heat in animals possessing at least one mutant allele (Dikmen et al., 2014, 2008; Littlejohn et al., 2014; Porto-Neto et al., 2018). These mutations cause a premature stop codon to occur and truncate the long form of the PRLR by 85 to 120 amino acids, thus removing two of the seven tyrosines involved in PRLR signaling in cattle (Littlejohn et al., 2014; Porto-Neto et al., 2018). Previously, the additional thermal benefits were thought to be as a result of the variation in coat length and thickness in slick cattle; however, due to the wide array of functions that prolactin possesses (Bole-Feysot et al., 1998), it is reasonable to hypothesize that the result of this improved thermal tolerance could be a result of something besides hair coat.

Through recent advances in genome editing technology, it is possible to introduce similar mutations into other species. Due to their short generation interval, low

cost, and the option of utilizing inbred strains (Arends et al., 2018), mice are commonly used in pilot studies before trials in other species or even humans. Additionally, the long form of the mouse PRLR has been shown to be present in the outer root sheath in mouse hair follicles. When the PRLR is knocked out in mice, the days to new hair growth significantly decreased for both males and females (Craven et al., 2001), suggesting PRLR plays a role in hair growth of mice just as it does in cattle.

Therefore, by heat stressing and analyzing hair regrowth in mice with a mutation that mimics the bovine slick mutation, we can begin to determine if this mutation can provide additional thermal benefits to other species and if a hair phenotype is present, as seen in slick cattle. This would ultimately allow us to conclude if the region associated with the slick mutation in cattle impacts mice in a similar fashion and provide a stepping point to incorporate this mutation into other species plagued by heat stress.

CHAPTER 2

LITERATURE REVIEW

INTRODUCTION:

Heat stress caused by increased ambient temperatures negatively affects many livestock industries. The total economic loss is estimated to be $1.7 billion across all animal species when heat abatement strategies are utilized. The swine industry constitutes $299 million of the total, beef and dairy industries contribute $1.26 billion, and poultry adds $128 million (St-Pierre et al., 2003). Further tactics are required to continue to lessen the burden associated with heat stress.

It is well documented that the bovine slick mutations provide superior thermotolerance to those animals with the mutation compared to their wild type (WT) counterparts. It is unclear whether the same mutation could bestow similar effects on other species. This chapter reviews the present literature on the use of mice as a model for livestock species, prolactin (PRL) signaling, the bovine slick mutation, and mouse thermoregulation.

MICE AS A MODEL SPECIES:

When designing research trials, cost is generally of concern. Due to this, rodent models are common for the initial trials of both human and livestock research. Mice are easier to handle, require less space, have a short generation interval, and are overall less

costly to maintain than livestock herds. These factors alone make mouse models valuable tools for the progression of knowledge.

Whole genome sequences are available for many species including cattle, swine, and sheep (Zerbino et al., 2017). This information allows researchers to understand the function of individual genes and/or gene interactions and for breed improvement through single gene selection or genomic selection. One of the most well characterized genomes is the mouse. Mice share anywhere between 92% and 95% of protein coding genes with cattle (Elsik et al., 2009), pigs (Humphray et al., 2007), and sheep (Iannuzzi et al., 1999). This is of importance when considering translational research such as the introgression of mutations naturally occurring in one species to another.

Another advantage to using mice is the option of using inbred strains to limit genetic variation. Classical inbred strains, such as the C57BL/6, were produced after 20 or more consecutive brother-sister matings (Green et al., 1963). Therefore, inbred lines reduce the genome to one allele variant per locus (Arends et al., 2018), eliminating genetic variation within the line. When investigating the effects of a genetic modification, minimal genetic diversity is desirable. For example, if an edited animal displays a phenotype not that of the wildtype, the variation can most likely be attributed to the edit considering the remaining genome should be identical. Lastly, by minimizing variation within a group of animals, the number of animals necessary for a given experiment will be reduced when compared to those with varying genetics, which is often the case in livestock species.

With this being said, there is no true way to know how a genetic modification, environmental stressor, or other experimental challenge will impact a livestock species

unless tested on that species specifically. When comparing livestock and mice there are varying anatomical, endocrine, and physiological differences that should be considered. These differences can impact behavior, fertility and productivity (Arends et al., 2018). Despite these downsides, mice remain a suitable subject for pilot studies prior to progressing to trials in larger species and/or humans.

PROLACTIN RECEPTOR:

Prolactin Receptor:

The prolactin receptor (PRLR) belongs to the class 1 cytokine superfamily of receptors (Kelly et al., 1991) and consists of three main domains: extracellular, transmembrane and intracellular. The extracellular domain is fairly conserved across species and across different cytokine receptors. This domain consists of approximately 200 amino acids and is often called the cytokine receptor homology region (Wells and de Vos, 1996). This region is further divided into two areas: D1 and D2 (Kelly et al., 1991). These regions help to drive ligand interactions as they are fibronectin type III modules (Wells and de Vos, 1996). Fibronectin is a cell adhesion molecule, and the type III domain in this receptor is thought to aid in ligand interaction (Kelly et al., 1991). Furthermore, there are two highly conserved structures in the ECD: two separate pairs of disulfide linked cysteines in the N-terminal region of D1 and a pentapeptide referred to as the WS motif. The WS motif is located in the membrane proximal region of the C-terminal region of D2 (Bole-Feysot et al., 1998). It is thought that the WS motif is necessary for correct folding and cellular trafficking (Goffin and Kelly, 1997).

The next segment of the receptor is the transmembrane domain. This domain is a 24

amino acid, single pass transmembrane chain. How this region is incorporated in signaling or receptor activity remains unknown (Bole-Feysot et al., 1998). Lastly, the intracellular domain has two quite conserved regions referred to as box 1 and box 2. Box 1 is in the membrane proximal region of the intracellular domain and is made of eight enriched proline residues. Current research suggests that this region has a folding sequence that is recognized by signal transducers. Box 2 consists of hydrophobic amino acids followed by negatively charged regions, then positively charged residues (Kelly et al., 1991).

Prolactin Receptor Isoforms:

The PRLR is capable of being produced in varying isoforms that differ in the length and sequence of their cytoplasmic tail. This is accomplished through alternative splicing of the primary transcript producing short, intermediate, or long forms of the receptor depending on the species. In all PRLR types, the extracellular domain is identical and the transmembrane domain remains similar (Goffin and Kelly, 1997). The main differences derive from the intracellular portion where box 2 is not present in the short isoforms of the receptor (Kelly et al., 1991).

In mice, one long and three short isoforms of the receptor have been identified (Clarke and Linzer, 1993; Davis and Linzer, 1989). Davis and Linzer discovered that in the mouse liver, there are three definitive murine PRLR which are now known as the short forms of the receptor. These receptors are indistinguishable when comparing their extracellular, transmembrane, membrane proximal cytoplasmic domains, and a 27 amino acid sequence in the cytoplasmic domain. The variation derives from unique sequences of 23 ($PRLR_S1$), 12 ($PRLR_S2$), or 30 ($PRLR_S3$) amino acids in the carboxy-terminal

region (Davis and Linzer, 1989). Shortly after this finding, the only known long form of the PRLR in mice was discovered in the ovary ($PRLR_L$) (Clarke and Linzer, 1993).

In cattle, there are also two forms of the PRLR: one long form (lPRLR) and one short form (sPRLR) (Schuler et al., 1997). Both long and short ovine PRLR cDNAs have been identified, which aided in the discovery of bovine PRLR due to their similar sequences. The short form is formed through alternative splicing when a 39 base insert at the beginning of the cytoplasmic domain causes two inframe stop codons (Bignon et al., 1997). There are a few known mutations that will impact the lPRLR in cattle. The major mutations being the slick mutations which cause truncations of the cytoplasmic domain (Littlejohn et al., 2014; Porto-Neto et al., 2018).

The main difference between the species is that in bovine sPRLR and mouse $PRLR_S1$, there is only one tyrosine residue in the cytoplasmic domain. Whereas murine $PRLR_S2$ and $PRLR_S3$ have three tyrosine residues (Schuler et al., 1997). Moreover, the number of tyrosines in the long forms of their receptors vary: bovine contain seven tyrosine sites (Schuler et al., 1997) and mice are known to have eight (Clarke and Linzer, 1993). Despite these differences, all long and short forms of the bovine and murine PRLR maintain the box 1 region, which has been identified as imperative for JAK2 activation, although the function of the short form has not entirely been elucidated in cattle (Schuler et al., 1997).

Prolactin Signaling:

The first step in the signaling cascade triggered by PRL is ligand induced activation occurring through homodimerization of the receptor. When PRL is bound to only one receptor binding site in the extracellular domain of the PRLR, the complex is

inactive. However, when PRL binds a second PRLR at site two, the homodimerization allows for signal transduction to initiate (Goffin and Kelly, 1997).

The next step in eliciting an effect from PRL involves the Janus Kinase (JAK) family. The cytoplasmic region of the PRLR cytoplasmic tail lacks intrinsic enzymatic activity; however, it is known that ligand binding to the PRLR results in tyrosine phosphorylation of JAK2. JAK2 is one of the four known members of the JAK family: JAK1, JAK2, JAK3, and Tyk2. JAK2 is constitutively associated to the PRLR. JAK2 associates in the membrane-proximal-cytoplasmic region, specifically box 1, allowing for the short isoform of the receptor to still generate physiological effects, though few are known (Goffin and Kelly, 1997). Box 1 contains a Src homology 3 (SH3) domain that is believed to aid in the association of the PRLR and JAK2, through an undefined mechanism (Bole-Feysot et al., 1998). SH3 domains are known to control protein interactions in regions where proline rich motifs are present, such as box 1 (Ren et al., 1993).

Although not yet elucidated, the current hypothesis behind the phosphorylation of both JAK2 kinases relies on ligand-induced homodimerization bringing the two kinases in closer proximity to one another. This would provide for the opportunity for cross-phosphorylation of the tyrosine residues on each kinase (Finidori and Kelly, 1995). Once the JAK is phosphorylated it allows for phosphorylation of tyrosine residues on the receptor itself. Unlike the long and intermediate forms of the PRLR, the short PRLR does not undergo tyrosine phosphorylation, although it contains tyrosine residues in the cytoplasmic domain (Bole-Feysot et al., 1998).

Signal Transducer and Activator of Transcription (Stat) proteins are imperative for

the PRL signaling cascade. Stat proteins contain five conserved domains: DNA binding domain, SH2 (SRC homology) domain, SH3-like domain, ubiquitous tyrosine, and C-terminal transactivating domain. Different forms of the Stat protein family are tied to PRLR signaling: Stat1, Stat3, and Stat5. Stat5 is the most commonly associated with PRL signaling due to its signaling role in mammary glands (Hennighausen et al., 1997). When unphosphorylated, Stats remain in the cytosol; however, when a receptor is activated by ligand binding, the phosphorylated sites on the JAK molecules become sites for Stat SH2 domain binding. This causes Stat phosphorylation and shortly after, Stat dissociation from JAK2. In order to enter the nucleus, Stat proteins must homodimerize or heterodimerize though the SH2 domain of one Stat interacting with the phosphotyrosine of the other Stat. From this point, the dimerized Stat proteins travel to the nucleus where they activate regions in promotors (Goffin and Kelly, 1997).

Prolactin's Role in Hair Growth:

Prolactin in many species has been shown to have some degree of effect on hair growth, especially species with seasonal cycles of pelage replacement in hair, such as sheep and goats (Celi et al., 2003; Nixon et al., 2002). Hair follicles are unique structures in the body regarding their continuous cycle of growth and regression. As with any cyclic process there are various stages of hair growth and development. The active growth portion of the hair cycle is referred to as anagen. Anagen is defined by hair shaft elongation, melanogenesis, and by keratinocyte proliferation. Catagen, the stage of follicular regression, is where apoptosis and terminal differentiation occur. This stage ultimately places the hair and follicle in a resting state known as telogen. The final stage is the shedding of the hair shaft and is referred to as exogen (Foitzik et al., 2003).

Previous research in a variety of species has indicated the PRL levels impact seasonal hair cycles by inducing both catagen and proanagen (Nixon et al., 2002; Pearson et al., 1996).

Prolactin affects seasonally dependent hair growth and pelage replacement. Seasonal changes in photoperiod, which impact circulating PRL levels, regulate the secondary fiber shedding of young cashmere bearing goats. In cashmere goats, molting generally occurs shortly after the winter solstice when photoperiod begins to increase. Prolactin levels also alter fiber growth and the beginning of fiber shedding in these goats. In a trial to clarify the link between PRL and hair growth in cashmere goats, circulating PRL was low from November to February and higher in March and April. The molt score recorded for this trial was positively associated with PRL levels, suggesting its role in the molting process (Celi et al., 2003).

Seasonal variation in PRL levels is well characterized in regard to its role in seasonal hair growth, but information on the PRLR's role in the process is less robust. In sheep, short day lengths suppress circulating PRL levels and long days trigger a severe rise in serum prolactin levels (Nixon et al., 2002; Pearson et al., 1996). During the wool follicle cycle, PRL appears to alter expression of the PRLR gene. When inducing wool growth, there was a decrease in PRLR mRNA with the rise in PRL suggesting receptor down regulation as a result of increasing PRL, thus reducing the signaling capabilities. During telogen and proanagen, PRLR mRNA levels were markedly higher, potentially causing germ cells of the hair to proliferate and form the inner root sheath and the hair fiber itself. Anagen was characterized as having low PRLR expression potentially due to the wool follicle already having been established at this time (Nixon et al., 2002).

In mice, synchronized waves of hair replacement, called molting, are not seasonal in contrast to the goats and sheep discussed previously. Molts initiate at the belly and then spread over the back as they progress towards the tail. The first molt begins at 22-28 days of age, and this allows for production of the next generation of hair (Craven et al., 2001).

The next study will discuss trials performed using null PRLR knockout (KO) 129SV mice. The KO targeted exon 5, in the extracellular domain. This exon, along with exon 4, contains a cytosine residue which is imperative for ligand binding and receptor activation (Ormandy et al., 1997). Using RT-PCR it was discovered that skin samples holding anagen hair follicles, contain transcripts for $PRLR_S2$, $PRLR_S3$, and $PRLR_L$. $PRLR_L$ was the most predominately expressed receptor in the samples. $PRLR_L$ was most expressed in the outer root sheath but was also found to be present in the sebaceous glands and the epidermis. In neonates, $PRLR_L$ was present, although at lower levels. Both short forms found in the adult were barely detectable in the neonates. $PRLR^{-/-}$ mice appear to have phenotypically identical coats however, when evaluated more closely variations existed in hair cycling. Overall, hair cycling was advanced in mice lacking functional PRLR. Females began to produce fibers at 33.0 days of age (DOA) in $PRLR^{-/-}$, and $PRLR^{+/+}$ produced new follicles at 61.9 DOA. Heterozygous animals had an intermediate phenotype with new follicles appearing at 50.1 DOA. In males, the time of growth was also significantly different but less dramatically than females, with $PRLR^{-/-}$ showing growth at 31.0 DOA and $PRLR^{+/+}$ producing new follicles at 34.9 DOA. The molting pattern remained unchanged regardless of genotype, but dramatic differences were observed due to gender (Craven et al., 2001).

Prolactin's Impact on Heat Perception:

Tall fescue toxicosis is caused by the consumption of tall fescue grass that has been infected with endophyte fungus. This fungus produces ergot alkaloids (predominantly ergovaline) that, although advantageous to plant performance, are damaging to livestock performance. The initial phenotypic symptoms of tall fescue toxicosis include heat stress, poor growth, rough hair coat (retention of winter hair coat into summer months), and poor reproductive performance. Upon deeper analysis, it was discovered that cattle grazing infected fescue also have significantly lower circulating PRL levels than those who consume uninfected fescue (Porter and Thompson, 1992).

Ergot alkaloids are structurally similar to both dopamine and norepinephrine due to their ergoline ring (Berde, 1980). Dopamine receptors can be bound by ergot alkaloids, eliciting an antagonistic effect on PRL and causing lower serum PRL levels (Floss et al., 1973). Additionally, ergot alkaloids are similar in structure to norepinephrine, which causes vasoconstriction. Sheep fed ergovaline positive fescue had no difference in circulating plasma norepinephrine levels (Elsasser and Bolt, 1987; Harmon et al., 1991). This suggests that because the norepinephrine levels have remained unchanged and vasoconstriction is occurring, that ergot alkaloids are also capable of binding to these receptors and provoking a physiological effect by triggering the same cascade as norepinephrine.

Dopamine antagonists have been proposed as a means of rescuing PRL levels. Metoclopramide (MC), a dopamine antagonist, has been fed to steers grazing endophyte infected tall fescue and improved performance in multiple scopes. As was expected when feeding a dopamine antagonist, serum PRL levels were rescued to levels seen prior

to feeding infected fescue. Additional improvements included supplemented steers grazed 22.4% of the time between 12:00-16:00 compared to 6.2% in untreated groups, and the average daily gain (ADG) of steers in the MC group surpassed that of the untreated group (Lipham et al., 1992).

In cattle exposed to ergot alkaloids, their ability to dissipate body heat is hindered due to an inability to conduct peripheral heat loss. The cause of this could be vasoconstriction of peripheral blood flow, retention of winter coats, or a combination of the two. In many studies cattle that stuffer from tall fescue toxicosis retain their rough winter hair coats into the summer (Hoveland et al., 1983; Schmidt and Osborn, 1993; Thompson and Stuedemann, 1993). Although the current literature does not illustrate a clear link between PRL and the retention of winter hair, mutations such as the 'hairy' mutation are caused by a mutation in the PRL hormone that prevents the formation of one of the three disulfide bonds, thus changing the structure of the hormone. As a result, these cattle have longer coats and more severe heat stress related symptoms including higher respiration rates, increased wallowing behavior, and increased rectal temperatures (Littlejohn et al., 2014). Additionally, a mutation in the PRLR has been shown to cause a "slick" hair phenotype (Littlejohn et al., 2014), and cattle of this genetic background appear to be less affected by the negative side effects of tall fescue toxicosis (Browning, 2004, 2002).

BOVINE SLICK MUTATION:

Origin:

At the beginning of the 20th century, the Senepol breed (*Bos Taurus*) was developed

on the island of St. Croix in the US Virgin Islands. Initially, the objective behind the development of the breed was to construct a polled animal with the meat producing ability of European breeds (Red Poll) while incorporating the tropical heat adaptations found in African cattle breeds. Recent genomic ancestry evaluations have shown that current Senepols are on average 10.4% Zebu, 0.6% West African taurine breeds, and 89% European breeds. The heat tolerance is believed to derive from either Zebu or West African influence or possibly both (Flori et al., 2012). Senepol cattle are distinct from typical, temperate adapted *Bos Taurus* breeds due to their exceptional thermotolerance when exposed to elevated ambient temperatures (Littlejohn et al., 2014). In recent years, slick-haired Senepols have been admired for their sleek and short hair coat with lower follicular density (Porto-Neto et al., 2018), making them phenotypically different from other *Bos Taurus* breeds. Their short coats appear to correlate with superior thermotolerance to elevated temperatures (Olson et al., 2003) and breeding other breeds such as Holstein and Angus, to Senepol/ Senepol crosses can provide improved heat tolerance to a level similar to Brahman cattle (*Bos Indicus)* (Mariasegaram et al., 2007).

Mutation:

The bovine slick mutation is caused by a single, dominant mutation (Olson et al., 2003) on chromosome 20, which contains the *slick* locus encompassing the prolactin receptor (Mariasegaram et al., 2007). The slick phenotype is caused by a premature stop codon (p.Leu462*). In exon 10, a single base deletion leads to a frameshift mutation causing the aforementioned stop codon. By introducing a stop codon, 120 C-terminal amino acids are lost from the cytoplasmic domain of the long isoform of the PRLR (Littlejohn et al., 2014). Since its discovery, another breed, Limonero, also has been

found to exhibit a slick phenotype. In this breed, three nonsense variants cause a premature stop codon to develop within the 11th exon of PRLR (Porto-Neto et al., 2018) which functions similarly to the p.Leu462* mutation described by Littlejohn and colleagues (2014). The premature stop codons occur only after the conserved N434 of bovine PRLR, meaning that each truncated sequence only contains five of the possible seven tyrosine residues after the transmembrane domain. All of the mutations also cause truncation to occur prior to the conserved Y512 of the PRLR (Porto-Neto et al., 2018).

In the lPRLR for cattle, there are two known clusters of alpha-helices and beta-stands in the extracellular domain that are able to contact the intracellular domain through the transmembrane domain. When the lPRLR is compared to the truncated lPRLR, one cluster of beta strands is absent for the slick lPRLR. It is unknown whether the missing beta-strands could influence dimerization of the PRLR when PRL is present and if this could be the reason for phenotypic differences observed between slick and WT cattle (Porto-Neto et al., 2018). Additionally, as started previously, two tyrosine residues are removed when the slick mutations are present as well. This alone, or combined with the missing beta-sheet cluster, could explain the phenotypes as well (Littlejohn et al., 2014; Porto-Neto et al., 2018). The short form of the PRLR appears to be unaffected by the mutations (Davis et al., 2017).

Evidence of Improved Heat Tolerance:

Hair weight per unit surface and coat thickness are central elements in heat dissipation for cattle (Bennett, 1964). Due to this, it is evident that slick animals are better suited to regulate body temperature than WT animals during heat stress conditions (Dikmen et al., 2008; Olson et al., 2003). To quantify the phenotypic difference in coat

density, Olson et al. took clipped hair weights from 25% Senepol calves. The calves were classified by hair length (HCT) ranging from 1 being slick and short hair to 4 indicating a dense, thick coat. Calves with an HCT 1 had significantly lower clipped hair weights than the weights of HCT 2, HCT 3, and HCT 4 calves. As a result, it was postulated that Senepols/ percentage Senepols were heat tolerant because the lack of hair would provide for easier evaporative heat loss and less heat would remain trapped on the skin, below the hair (Olson et al., 2003).

To assess if the mutation had impacts beyond coat density, Dikmen and colleagues crossed Holstein cows to 25% Senepol 75% Holstein bulls that were heterozygous for slick. The offspring from these matings and their subsequent offspring were used in a variety of heat stress related trials. When lactating slick Holstein cows were subjected to heat stress conditions, respiration rates increased less drastically, rectal temperatures (RT) underwent a less severe increase (Dikmen et al., 2014), and vaginal temperatures remained lower (Dikmen et al., 2014, 2008) than wild-type cows. In percentage Senepol calves, similar results ensued. Lower RT and breaths per minute (BPM) were found in calves with the slick allele when compared to WT haired calves. Additionally, calves of identical breed composition but that vary in their coat scores (slick or hairy) showed variation in weight gain. The slick calves gained significantly more weight (13 kg) over a six-month period (Olson et al., 2003). Prior to the identification of the slick mutation, a study was done with Hereford, Senepol and Hereford x Senepol crossbred cattle. The results showed that Senepol cattle grazed longer, especially during warmer times of the day, and maintained a lower rectal

554 temperature during these elevated temperatures (Hammond and Olson, 1994), providing
555 an explanation for the superior weight gain in slick animals.

556 These advantages appear to be limited to those animals with coat scores of one,
557 due the mutation being dominant. For example, Angus sired 25% Senepol calves with
558 coat scores of two or greater did not differ from purebred Angus calves when comparing
559 RT in summer months. Even when breed composition was identical, normal haired
560 offspring consistently had higher RT than slick calves (Olson et al., 2003). Furthermore,
561 when WT cattle coats were clipped to similar hair length as slick cattle, their RT were
562 lower than the unshaved animals, yet still above the that of the slick cattle (Hammond
563 and Olson, 1994). This finding alludes to an underlying physiological mechanism
564 beyond differences in coat density could be involved in increasing thermotolerance of
565 slick cattle.

566 Sweating rate differences have been a source of inconsistency when comparing
567 studies pertaining to slick cattle. Sweating rate tended to be higher when measured at the
568 shoulder in slick animals providing for increased evaporative heat loss (Dikmen et al.,
569 2008). This innate ability to sweat more readily is of benefit when taking into account
570 that 85% of heat loss in dairy cattle is evaporative (Maia et al., 2005). However, this
571 advantage was dissipated when hair was clipped as slick cows' sweating rates were not
572 different from WT cows' rates in clipped areas. As a result, it was hypothesized that this
573 higher sweating rate with short hair was due to the less humid air being caught at the
574 skin's surface because removing the hair would eliminate this advantage, thus yielding
575 equal perspiration rates (Dikmen et al., 2008). Dikmen and others 2014 study contradicts
576 the previous hypothesis because sweating rate remained higher at the neck, loin, rump,

and hind leg in slick cattle compared to WT even when shaved. Ergo, it was postulated that the increased sweating was due to a greater sweat gland density in slick cattle or that slick sweat glands more readily produced sweat.

MOUSE THERMOREGULATION:

Maintaining a stable core body temperature when exposed to environmental temperatures outside of the thermoneutral (TN) zone is a critical task homeotherms must accomplish to maintain homeostasis (Gordon and Jong, 1984). During heat stress specifically, heat exchange between the body and environment must increase while bodily heat production must decrease to avoid hyperthermia (Terrien et al., 2011). Depending upon the strain, mice preferred ambient temperature is 26° to 30°C (Gaskill et al., 2011, 2009; Gordon et al., 1998). The maintenance of this temperature is generally accomplished by both autonomic and behavioral effectors that are sensitive to changes in ambient temperature (Gordon and Jong, 1984).

<u>Autonomic:</u>

The most common forms of autonomic thermolysis behaviors are sweating, panting, and vasodilation (Terrien et al., 2011); however, mice do not sweat or pant, therefore vasodilation will be the focus of this section. Changes in peripheral vasomotor tone (PVMT) are known to aid abatement of dry heat through conductive and convective mechanisms. This involves shunting warm blood from the core into the peripheral tissues that generally lack insulation and fur. The heat loss is facilitated through the autonomic control of smooth muscle tone in arteriovenous anastomoses (AVAs) and arteriolar precapillary sphincters that cause vasodilation. In the rodent the main location that

PVMT controls heat loss is in the tail (Gordon, 1993).

The majority of tail thermoregulatory work has been done in rats with little mouse work having been done in this field, though researchers tend to accept that a similar pattern should uphold in the mouse. In both mice and rats, the tails are relatively hairless when compared to the rest of the body and have a high density of AVAs (Gemmell and Hales, 1977). This provides for an ideal place to accelerate blood flow to during heat stress situation, and with the high surface area to volume ratio, heat dissipation is efficient. Past research has shown that if the tail is amputated, when given thermogenic drugs, higher body temperatures result along with a lower heat tolerance (Spiers et al., 1981). In rats, at room temperatures ranging between 20-25°C (TN), blood flow to the tail is extremely low, nearing zero. When the temperature rises outside of TN, blood flow to the tail increased by approximately 10 times (Rand et al., 1965). Additionally, if ambient temperatures exceed tail skin temperature, increased blood flow actually adds heat to the body as it accepts heat from the external air. Rats appear to have a threshold of roughly 35°C where tail blood flow increases until this temperature and then steadily decreases when external temperatures exceed this point (Raman et al., 1983). The only direct research in this field with tail blood flow in mice was done by Gordon. His finding suggesting that when exposed to radio frequency radiation, tail blood flow markedly increases due to the increasing heat (Gordon, 1983).

<u>Behavioral:</u>

When given a gradient of temperatures to reside in, most mammals have a set range of temperatures they tend to stay within. The act of the organism moving to their preferred temperature is a foundation of behavioral thermoregulation. The temperature

range the animals prefer to stay in is referred to as their "thermal preferendum" (Reynolds and Casterlin, 1979). The central nervous system is capable of integrating the core temperature data and trigger various responses to aid in thermoregulation (Van Someren et al., 2002). Mammals tend to use behavioral effectors to reach their desired temperature rather than autonomic effectors because most often, they require less energy to accomplish. The most common method of behavioral thermoregulation in C57Bl/6 mice is thermotaxis (moving to a more suitable temperature) (Gaskill et al., 2012). However, in laboratory rodent housing conditions stable temperatures are maintained, and if exposed to heat or cold stress, other behavioral effectors will need to be triggered.

In states of heat stress, decreasing locomotive energy expenditure and heat production is imperative in many species. A common mechanism of heat dissipation seen in rodents involves the animals assuming a prone position and extending their extremities as far as possible. This helps to maximize the surface area to body mass ratio and thus increases heat loss (Terrien et al., 2011).

Energy intake also correlates to body heat production. High ambient temperatures reduce the need for energetic body heat production, thus reducing caloric needs. It has been shown in humans, rats, and piglets that decreasing feed intake is a common mechanism to control body heat production (Brobeck, 1948; Collin et al., 2001; Westerterp-Plantenga, 1999). On the contrary, mice subjected to cold stress need to consume more feed to maintain their body temperatures. In order to maintain their body temperature, mice start to use more energy for thermogenesis and consequently eat approximately 2 grams more per mouse at 20°C as they would at 30°C (Cannon and Nedergaard, 2009).

One of the simplest ways to asses behavioral thermoregulation in mice is through nest structure. Christopher Gordon's lab has done extensive work looking at the effect ambient temperature has on nest structure. The group placed mice into cages with two nestlets. Well-built and structured nests were observed at temperatures between 22°C and 30°C. At 22°C mice were hidden under the nestlet material, presumably because this is below their TN temperature. At TN (30°C), the mice remained in their nest but uncovered. By increasing the temperature to 32°C, mice began to breakdown their nests but remained in contact with the nesting material. At 34°C the nests were entirely unorganized, suggesting the mice were experiencing heat stress (Gordon, 2017). Gaskill and colleagues performed a similar experiment with C57Bl/6 mice where nest score was recorded on a 1-5 scale with 1 being poor and disorganized and 5 being highly structured. Nest scores decreased in a linear fashion as room temperature increased from 20°C to 25°C and then to 30°C. Interestingly, there was an effect of gender in this process with males and females having similarly scored nests at 20°C. While at 25°C and 30°C, females had significantly higher nest scores (Gaskill et al., 2011).

Gender:

Finding differences in thermoregulation due to gender is thought to derive from how the hypothalamic preoptic area is sexually dimorphic, but this area is also vital in temperature homeostasis (Sanchez-Alavez et al., 2011). In humans, on multiple occasions, males and females have been shown to vary in thermoregulatory capability with females producing less sweat when exposed to elevated ambient temperatures (Kaciuba-Uscilko and Grucza, 2001) and not initiating thermoregulatory responses until a higher core body temperature was obtained (Lopez et al., 1994).

669 In C57Bl/6 mice specifically, at 25°C differences in core body temperature are
670 apparent. In young mice (defined as 3 months of age), the females had a varying pattern
671 of both core temperature and locomotor activity when in estrus. When compared to
672 males, females had a core temperature 0.2-0.5°C higher and had 30% more locomotor
673 activity in the dark phase. In the aged group, resting core temperature was significantly
674 higher in the females (0.6°C), but this difference was eliminated during the active period
675 (Sanchez-Alavez et al., 2011). It has also been postulated that the sexual dimorphism
676 seen in C57Bl/6 mice, with females being generally lighter than males, could alter their
677 thermal preference. Females that are lighter would have a higher surface area to volume
678 ratio which would increase their ability to lose heat to the environment (Gaskill et al.,
679 2009; Gordon, 1993).
680 Age:
681 Increasing age tends to hinder thermoregulatory abilities during hypothermia and
682 hyperthermia in many species, the most well documented instances being in humans (Rey
683 et al., 2007; Tanaka and Tokudome, 1991). In humans, dehydration risks increase when
684 undergoing heat stress (Van Someren et al., 2002) due to evaporative cooling
685 inefficiencies that seem to derive from increasing age (Collins and Exton-Smith, 1983).
686 In C57Bl/6 males and females, age is associated with variation in
687 thermoregulatory ability when compared to younger animals. At 24 months of age,
688 females do not undergo the same circadian profile of temperature variation caused by the
689 estrous cycle that the 3-month-old females experience. Also, when mice enter the dark
690 stage (active stage), their core temperature increases. In aged males and females, it took
691 three to four times as long to reach their dark stage core temperature as it did for the

692 younger group. However, final temperature obtained was not different when comparing
693 young to aged within each sex. Differences during the light phase were not present
694 (Sanchez-Alavez et al., 2011).
695 Dynamic Temperature Regulation:
696 Most mammals are considered to be homeothermic, meaning they are capable of
697 maintaining a stable core body temperature over a wide range of ambient temperatures
698 (Gordon and Jong, 1984). Recent improvements in thermoregulatory technology, such as
699 implantable data loggers, provided insight that shows mice do not necessarily fit the
700 general homeothermic pattern when looked at in short term segments (1-60 minutes),
701 although over multiple hours they average to approximately 36°C temperature (Gordon,
702 2009).
703 In a 2009 study, Gordon implanted mice with radiotelemetry devices located in
704 their peritoneal cavities that recorded body temperatures every minute over a 24-hour
705 period. The study demonstrated that C57Bl/6 mice's body temperatures can vary by
706 2-4°C (Gordon, 2009). Whereas studies with rats (Schmidt and Osborn, 1993), elephants
707 (Kinahan et al., 2007), and humans (McKenzie and Osgood, 2004) all showed no more
708 than a 2°C range from the highest to lowest recorded body temperatures. Therefore,
709 although the average core body temperatures across these species are relatively similar,
710 all within approximately 3°C of each other (Gordon, 2012), the range of core temperature
711 they withstand before sparking significant central nervous system responses varies
712 greatly and alludes to differences in thermoregulatory responses. Additionally, rats have
713 been shown to be better thermoregulators in terms of maintaining a more consistent core
714 temperature with temperature differentials (changes in temperature from one time point

to the next) of smaller magnitude compared to mice, presumably due to the difference in body size between the species (Gordon, 2009). Once again attesting to the idea that larger species require more thermal inertia to increase their body temperature, thus their core temperature is much less variable than that of a small rodent (Gordon, 2012).

CONCLUSIONS:

The goal of this review was to describe the importance of mice as a model species, the process of PRLR signaling, the bovine slick mutation, and thermoregulation in the mouse. The bovine slick mutation has been shown to provide added thermotolerance to those that carry at least one mutant allele for the gene. Although typically it is believed to provide benefits due to the less dense hair coats these cattle possess, additional underlying physiological mechanisms may also be changed. Additional research to understand if a similar truncation would yield similar improved thermotolerance during elevated temperatures in other species would potentially be of great benefit to other industries that are plagued by heat stress related problems.

CHAPTER 3

RESPONSE OF MICE WITH THE BOVINE SLICK MUTATUION TO THERMAL STRESS

INTRODUCTION:

The bovine slick mutation has been repeatedly shown to improve thermotolerance to heat in cattle possessing at least one mutant allele (Dikmen et al., 2014, 2008; Littlejohn et al., 2014). Many livestock species experience the detrimental impacts that heat stress has on productivity, and it costs livestock industries millions of dollars yearly even when heat abatement strategies are implemented (St-Pierre et al., 2003). Through advances in genome editing technology, it is possible to create similar mutations in other species. Due to their short generation interval, low cost, and the option of utilizing inbred strains (Arends et al., 2018), mice are commonly used in pilot studies prior to trials in other animal species or humans. Therefore, by heat stressing mice with a mutation that mimics the bovine slick mutation, we can begin to determine if this mutation might confer additional thermal benefits to other species as we consider incorporating this mutation into other species to confer heat tolerance.

MATERIALS AND METHODS:

Animals and Facilities:

All animal procedures were approved and reviewed by the University of Missouri Animal Care and Use Committee (Protocol #9552). C57Bl/6 wild type (WT/WT) mice

were obtained from Jackson Laboratories (Bar Harbor, ME). Genetically modified C57Bl/6 founder males were obtained from the Animal Modeling Core at the University of Missouri. These mice had a mutation that mimicked the mutations found in slick cattle. Founder males were mated to WT/WT C57Bl/6 females to produce heterozygous (WT/MUT) offspring. These offspring were then mated to produce homozygous mutant (MUT/MUT) pups. Mice were housed in transparent acrylic cages with ground corn cob bedding and a single nestlet (Ancare, Bellmore, NY) per cage. Males were housed individually, and breeding females were group housed with a maximum of four mice per cage. At three weeks of age, pups were weaned, ear notched for identification, and tail snips (less than 5 mm) were collected for genotyping. From three to six weeks of age littermates of the same sex were group housed with a maximum of four mice per cage. Mice were housed at 23 ± 1°C and were on a 12:12 L:D photoperiod. Feed (5001 Rodent Diet; Lab Diet®, Brentwood, MO) and water were provided *ad libitum*.

Experimental Design:

When mice reached six weeks of age, they were moved to the environmental chambers in Unit B of the Animal Science Research Center (University of Missouri, Columbia, MO). Ambient temperature and humidity data were recorded every 15 minutes using Pro V2 Hobologgers ® (Onset Computer Corporation, Bourne, MA, USA). Mice were individually housed in transparent acrylic cages with ground corn cob bedding material and a single nestlet per cage. Feed (5001 Rodent Diet; Lab Diet, Brentwood, MO) and water were provided *ad libitum*.

Prior to entering the environmental chambers, body weights were recorded, and genotypes were determined using the protocol described below. Body weights were

776 recorded once more when exiting the chamber after 10 days. Mice underwent a series of
777 3°C ambient temperature increases every other day starting at 22°C and concluding at
778 34°C (10d) (Figure 3.1). Temperatures were increased at 12:00 ± 1 hour or 20:00 ± 1
779 hour. Daily measurements included food disappearance (FD), water disappearance using
780 25-mL serological pipette waterers (Haag et al., 2018), tail temperature (TT) and nest
781 score (NS) (Hess et al., 2008). TT was recorded with an infrared temperature gun
782 (Raytek, Evertt, WA, USA) at the base of the tail. Nesting behaviors were appraised
783 visually each day as a behavioral indicator of thermal stress. Additionally, for the
784 temperature increase from 28°C to 31°C nest scores were recorded once hourly, for three
785 hours, to assess to progression of nest destruction.
786 Genotyping:
787 After tail snips were obtained, they were cut into smaller pieces, added to 100 µL
788 of embryo lysis buffer (40mM Tris, pH 8.9; 0.9% Triton X-100; 0.9% Nonidet P-40), and
789 one µL of proteinase K. Then, they were incubated at 65°C for 60 minutes to disrupt
790 cells and raised to 95°C for 15 minutes to inactivate proteinase K. One µL of tail lysate
791 was used as template in a 25 µL PCR volume with the following parameters: 94°C initial
792 denaturation for 30 s, followed by 30 cycles of a 15 s denaturation at 94°C, 30 s
793 annealing at 60°C, and 1 min extension at 68°C, with a 68°C final extension for 5 min.
794 The PCR consisted of 5 µL of one taq buffer, 0.5 µL of DNTPs, 0.25 µL of one taq hot
795 start, 16.25 µL of water, and 1 µL of each the forward
796 (5'CATCCCTGAGATCACTGAGAAGCC 3') and reverse
797 (5'TGGCATACTCCTTACTGGTTTCAGG 3') primers.

Lastly, a digestion consisting of 5 µL of PCR product, 1 µL of AflII, 1 µL of Cutsmart buffer, and 13 µL of water was mixed. This incubated at 37°C overnight. The reaction product was then run on a 2% agarose gel using TBE buffer. The ladder comprised 0.5 µL pBR322 DNA MspI, 1 µL purple gel loading dye, 1 µL Cutsmart buffer, and 7.5 µL water. Following the digestion three band patterns could be observed: a WT/WT mouse has a single 491 bp band, a MUT/MUT mouse has two bands (not distinguishable from one another on a gel) at 242 bp and 249 bp indicating a cut, mutant allele, or a WT/MUT mouse has one 491 bp WT band and a 242 bp and 249 bp band (Figure 3.2).

Topo cloning was performed using the Topo 2.1 cloning vector and protocol from Invitrogen (Carlsbad, CA). The PCR product from the protocol above was included as the template DNA. Samples were sent to the DNA Core following the completion of Topo 2.1 protocol. To determine which of the initial mouse pups potentially possessed the desired mutation, samples were sent to the DNA Core at the University of Missouri to perform Sanger DNA sequencing.

Line Selection:

The Animal Modeling Core produced eight founder males potentially containing mutations similar to the bovine slick mutation. Considering the bovine slick mutations cause a premature stop codon in a 36 amino acid region of the PRLR cytoplasmic domain, lines with mutations in the corresponding region the mouse genome (Figure 3.3a) not involving this stop codon or mutations outside this region were not pursued nor discussed in this paper. The two lines with the desired stop codon were line 003 and 259 (Table 3.1). However, due to an unforeseen circumstance, mouse 259 had to be

821 euthanized due to a tumor developing and his offspring died shortly after weaning, thus
822 eliminating this line as a viable option. Therefore, the remainder of this paper will focus
823 on the descendants of line 003. The amino acid sequence for line 003 was compared
824 back to the mouse WT/WT sequence (Figure 3.3b) and the bovine amino acid sequence
825 (Figure 3.3c) to confirm the mutation was in the slick, target region.
826 Statistical Analysis:
827 Data collected throughout the heat stress trials were analyzed using the
828 generalized linear mixed model procedure of SAS (PROC GLIMMIX). Data was
829 collected once daily and included the following variables: FD, WD, TT and NS. Due to
830 the relationship between feed and water consumption in laboratory rodents fed pelleted
831 diets (Kraly, 1984), water disappearance per unit of feed disappearance (W/F) was
832 calculated. Due to substantial skewedness in these data, the log function in SAS was
833 taken to make the outputs more symmetrical for WD and W/F.
834 The model included the main effects of sex, genotype, and room temperature. All
835 interactions were also evaluated. Weight was included in the model and corrected for in
836 each response variable. Data are presented as least squares means ± standard error of the
837 least squares mean. Means were considered significant at $P<0.05$ and considered to have
838 a tendency toward significance if $0.05 \leq P \leq 0.10$.
839 Data collected during the temperature increase from 28°C to 31°C were analyzed
840 using the general linear model procedure in SAS (PROC GLM). For each mouse, one
841 nest score was recorded per hour for three hours following the initiation of the
842 temperature increase. The main effects of genotype, sex and time of temperature increase
843 (AM or PM) were included in the model. All interactions were also included. Data are

presented as least squares means ± standard error of the least square mean. Means were considered significant at $P<0.05$ and considered to have a tendency toward significance if $0.05 \leq P \leq 0.10$.

RESULTS:

Feed Disappearance:

All two-way interactions (sex by genotype, sex by temperature, and genotype by temperature) significantly impacted FD ($P<0.05$) (Table 3.2). In the sex by genotype interaction, significant differences are observed ($P=0.0041$): female MUT/MUT (3.49 ± 0.08) vs. male MUT/MUT (3.21 ± 0.09), female WT/WT (3.36 ± 0.05) vs. male WT/WT (3.55 ± 0.06), male WT/WT vs. male WT/MUT (3.38 ± 0.04), and male WT/WT vs. male MUT/MUT (3.21 ± 0.09) (Figure 3.4a). The sex by temperature interaction model was also significant ($P<0.0001$). In this interaction, males and females at the same temperature for 22°C, 25°C, 28°C and 31°C did not differ ($P>0.05$) except at 34°C females had a significantly higher FD than males (Figure 3.4b).

Lastly, the genotype by temperature interaction shows that MUT/MUT (4.13 ± 0.12) and WT/MUT (4.19 ± 0.06) had substantially lower FD at 22°C than WT/WT (4.41 ± 0.08) ($P<0.05$). At 25°C MUT/MUT mice (3.78 ± 0.10) had a lower FD than WT/WT (4.06 ± 0.06) ($P<0.05$) but did not differ from WT/MUT (3.90 ± 0.05) ($P>0.05$). Additionally, WT/MUT no longer differed from WT/WT mice at 25°C ($P>0.05$). At both 28°C and 31°C no differences were observed among the genotypes ($P>0.05$). At the highest temperature, WT/MUT (2.55 ± 0.03) and MUT/MUT (2.60 ± 0.05) had higher FD than WT/WT mice (2.43 ± 0.03) ($P<0.05$) (Figure 3.4c).

Water Disappearance:

The sex by temperature interaction significantly impacted WD ($P<0.05$) (Table 3.2). In this interaction, males and females do not differ at 22°C, 25°C, and 28°C ($P>0.05$) but diverge thereafter with females having a significantly higher water disappearance than males at both 31°C and 34°C ($P<0.05$) (Figure 3.5).

Water Disappearance per unit of Feed Disappearance:

The two-way interactions for sex by temperature and genotype by temperature significantly impacted W/F ($P<0.05$) (Table 3.2). In the sex by temperature interaction, no differences between the sexes were observed at 22°C or 25°C ($P>0.05$). As the temperature continued to increase, the females had a significantly higher W/F ratio compared to males at 28°C, 31°C, and 34°C ($P<0.05$) (Figure 3.6a). When evaluating the genotype by temperature interaction, the opposite occurs with no differences occurring among genotypes at 28°C, 31°C, and 34°C ($P>0.05$). However, at 22°C both the WT/MUT and MUT/MUT had a higher W/F ratio than WT/WT ($P<0.05$). At 25°C, MUT/MUT and WT/WT remain different ($P<0.05$) with WT/MUT having an intermediate ratio (Figure 3.6b).

Tail Temperature:

The main effects of sex, genotype, and temperature were significant for TT ($P<0.05$) (Table 3.2). Males and females showed different TT during the trial (F= 28.84 ± 0.0.05 vs. M= 29.07 ± 0.06; $P<0.05$). When analyzing the differences due to genotype, WT/MUT mice had warmer TT than MUT/MUT mice (WT/MUT= 29.07 ± 0.04 vs. MUT/MUT= 28.81 ± 0.08; $P<0.05$) and WT/WT mice had an intermediate, statistically indifferent TT in respect to WT/MUT and MUT/MUT. Lastly, room temperature had a

drastic impact on TT with all room temperatures being significantly different from each other ($P<0.0001$) (Table 3.3).

Nest Score:

The three-way interaction among sex, genotype and temperature was significant for NS ($P<0.05$) (Table 3.2). At 22°C, male WT/WT had significantly a lower NS than male MUT/MUT (WT/WT M= 4.31 ± 0.12 vs. MUT/MUT M= 4.78 ± 0.15; $P<0.05$). Additional differences were found between WT/MUT males and MUT/MUT females (WT/MUT M= 4.58 ± 0.10 vs. MUT/MUT F= 4.15 ± 0.14; $P<0.05$) and MUT/MUT females compared to MUT/MUT males (MUT/MUT F= 4.15 ± 0.14 vs. MUT/MUT M= 4.78 ± 0.15; $P<0.05$). At 25°C the only significant differences were between WT/WT males (3.72 ± 0.15) and both WT/MUT males (4.11 ± 0.12) and MUT/MUT males (4.22 ± 0.19), in which WT/WT mice build significantly poorer nests than their counterparts ($P<0.05$).

Male WT/WT built significantly poorer nests than WT/MUT males, WT/MUT females, and MUT/MUT females at 28°C (WT/WT M= 3.07 ± 0.16 vs. WT/MUT M=3.86 ± 0.13, WT/MUT F=3.60 ± 0.15, and MUT/MUT F= 3.56 ± 0.18; $P<0.05$). Additionally, at 28°C, WT/MUT males differed with WT/WT females and MUT/MUT males, in which WT/MUT males had more well-constructed nests (WT/MUT M= 3.86 ± 0.13 vs. WT/WT F= 3.38 ± 0.14 and MUT/MUT M= 3.32 ± 0.20; $P<0.05$).

There were still differences between genders at 31°C for male WT/WT versus all three female genotypes ($P<0.05$). The only difference within the same gender male WT/WT versus male MUT/MUT in which MUT/MUTs had significantly higher NS at 31°C than WT/WT (WT/WT M= 1.07 ± 0.13 vs. MUT/MUT M= 1.53 ± 0.16; $P<0.05$).

Lastly, at 34°C no differences were observed among any of the genotype, sex combinations (P>0.05) (Figures 3.7 A and B).

Time at the 28°C to 31°C Temperature Increase's Effect on Nest Score:

For hour 0, the beginning of temperature increase, the effects of sex, genotype, time of temperature increase, and all interactions were insignificant (P>0.05). For the hour 1, hour 2, and hour 3 sex and genotype continued to have no effect on NS (P>0.05); however, time of temperature increase strongly affected nest score (P<0.0001). Similar to hour 0, all interactions were nonsignificant for hour 1, hour 2, and hour 3 (Figure 3.8).

DISCUSSION:

Feed and Water Disappearance:

Heat stress commonly results in decreased feed intake and increased water intake in many species (Brobeck, 1948; Collin et al., 2001; Dale and Fuller, 1980; Rhoads et al., 2009; Westerterp-Plantenga, 1999). W/F should also be considered in this analysis because of the relationship between feed and water in rodents (Kraly, 1984).

In both FD and W/F, no differences among genotypes were observed at 28°C or 31°C suggesting that at temperatures near or slightly exceeding TN that the genotypes behave similarly. This is consistent with what is observed in slick and WT cattle, with differences only being viewed at heat stressed temperatures. At 34°C, feed disappearance is significantly higher in those animals with at least one copy of the mutant allele, indicating that they are less bothered by the higher ambient temperatures due to their ability to consume more feed than WT/WT mice. Additionally, there is a tendency for the MUT/MUT to have a lower W/F ratio at 34°C versus WT/WT . The combination of the

936 FD and W/F data allude to improved thermal tolerance as MUT/MUT animals are
937 consuming the most feed while maintaining the lowest W/F ratio at 34°C showing they
938 are not consuming excessive water to aid in heat abatement.
939 Interestingly, there are also stark differences among the genotypes at the lowest
940 temperatures (22°C and 25°C), designed to be below their TN temperature zone. The
941 current literature for cattle with the bovine slick mutation only focuses on heat stress, not
942 cold stress. However, the data presented today shows that those mice with the slick
943 mutation are also behaving in a different manner than WT mice are at low temperatures.
944 WT/WT mice have the highest FD at both 22°C and 25°C and MUT/MUT have the
945 lowest. This could mean a few things or a combination of the two. Mice whom are cold
946 stressed are known to eat approximately 2 grams more than they do at TN (Cannon and
947 Nedergaard, 2009) in order to maintain their body temperature. It appears that the
948 WT/WT mice are bothered by the low temperature due to their increased FD. For the
949 W/F ratios during this time, MUT/MUT consumed significantly higher ratio than
950 WT/WT and WT/MUT remained the intermediate phenotype. Although water
951 disappearance at temperatures below TN is not well described in mice, one could assume
952 that the mice with higher W/F ratios had this effect because they were more active
953 outside of their nests rather than spending time in their nests attempting to warm
954 themselves thus resulting in higher water consumption. Overall, this mutation appears to
955 be involved in thermal perception and/or thermoregulation at temperature extremes, but
956 does not impact the mice at TN.
957 Differences in in the sex by temperature interaction were present in the models for
958 FD, WD, and W/F. At the 34°C, females had higher a FD than males suggesting that

959 they are more heat tolerant due to their ability to consume more feed at higher ambient
960 temperature. This is consistent with recent research that alludes to female mice being
961 more heat tolerant than males. In a study by Garcia and colleagues regarding exertional
962 heat stroke, when exercise initiates, female mice better maintained their core temperature,
963 ran for longer prior to stroke, and obtained a higher maximum speed. Part of this is
964 thought to be as a result of the variation in body mass to surface area ratio between males
965 and females, with the advantage aligning with the females in terms of heat abatement
966 (Garcia et al., 2018). Another study confirms the theory in which female mice have a
967 higher heat dissipation rate due to their higher body surface area to body mass ratio
968 (Kaikaew et al., 2017). This should allow them to consume more food at the higher
969 temperatures due to their bodies allowing for more passive heat loss than males.
970 The variation between genders at 34°C in FD could partially cause the difference
971 found in WD at 34°C because the additional FD compared to males would require more
972 water to rehydrate. However, at 31°C females also had statistically more water
973 disappearance without a difference in FD. Additionally, this increase in water
974 consumption directly contradicts the suggestion that females are more heat tolerant than
975 males. The W/F data further opposes the conclusion that females are less bothered by
976 elevated temperature as their W/F ratios are statically higher than males at 28°C, 31°C
977 and 34°C. Although initially this information lends to females being less heat tolerant, it
978 could suggest that females are using this water as a heat abatement strategy and therefore
979 allowing them to be more heat tolerant. Mice have higher evaporative heat loss in
980 elevated ambient temperatures. At these high temperatures, their grooming pattern
981 changes with most of their grooming time being focused on their heads rather than their

bodies, in direct contrast to what is observed at thermoneutral temperatures. Although overall grooming decreased at elevated temperatures, it was hypothesized that while grooming more saliva was being secreted hence the increased evaporative water loss (Roberts et al., 1974). Contrary to mice, rats do produce excess saliva solely to wet the fur on their bodies more thoroughly to increase heat dissipation through evaporative cooling; however, this same behavior is not observed in mice (Yanase et al., 1991), simply a change in grooming pattern is noted. Mice generally opt for assuming an extended body position, to facilitate heat loss (Gordon, 1993) and have also been described displaying "escape behavior" from their cages when temperatures exceed 37°C, presumably looking for a cooler environment (Harikai et al., 2004). Overall due to the ample research indicating that female mice are more adaptable to warmer temperatures (Gaskill et al., 2011, 2009; Kaikaew et al., 2017), it is reasonable to conclude that the females in this study are more heat tolerant and use water to aid in coping with the elevated ambient temperatures thus increasing WD and W/F.

Tail Temperature:

When considering the use of infrared tail measurements as indicators of body temperature, it is important to remember that this only truly provides data on the tail vasculature not the entire the body. The tail in rodents is also known to be an area of thermoregulation where blood is shunted to help cool the animal during heat stress (Gordon, 1983; Rand et al., 1965). In this study, there was an overall effect of temperature in which each successive ambient temperature caused TT to increase as well. This is to be expected as mice use their tails to cool themselves and would be shunting

more blood to their tails at the higher temperature (Gordon, 1993), thus increasing their TT.

Females had a lower average TT over the course of the trial. Although the exact physiology behind why this might occur is unclear, a few potential explanations stem back to the general trend that females tend to take longer prior to triggering a response due to heat stress, and the difference in thermoneutral temperature when compared to males. Due to females generally being able to maintain their core temperature longer than males in elevated temperatures, this might indicate that females would be shunting less blood to their tail for heat loss (Garcia et al., 2018; Gordon, 1993; Lopez et al., 1994). Furthermore, recent studies have shown that female mice prefer warmer ambient temperature than males (Gaskill et al., 2011, 2009; Kaikaew et al., 2017), which would also suggest that at temperatures such as 31°C, presumably females are not as stressed and thus do not need to use their tails to facilitate heat transfer and maintain their core temperatures. Lastly, as noted previously, female C57Bl/6 mice are generally lighter than males causing them to have a higher surface area to body mass ratio (Gaskill et al., 2009; Gordon, 1993) which would increase their ability to lose heat to the environment and not cause their TT to increase as severely.

The interaction between genotypes is the most perplexing considering that WT/WT animals are intermediate to WT/MUT and MUT/MUT allowing for the only significant difference to be between WT/MUT and MUT/MUT. The TT results indicate that having both copies of the allele is necessary to alter thermotolerance. The results show that MUT/MUT animals have a lower overall TT, potentially indicating that at higher temperatures MUT/MUT are better suited to tolerate the thermal stress and are not

using their tails to eliminate as much heat as the other genotypes. This is also in contrast to what is observed in cattle because animals with only one copy of the allele show improved thermotolerance (Dikmen et al., 2008). Arguably, implantable core temperature loggers would have provided a sounder measure of whether mice with the introduced mutation were better able to maintain a lower core temperature over the course of the heat stress trial with only one copy of the mutant allele.

Nest Score:

When evaluating the three-way interaction between sex, genotype and temperature, it becomes clear that there are no differences among females when comparing the genotypes at any of the temperatures. Males have genotypic differences at all temperatures besides at 34°C where all nest scores are nearing 1, which is indicative of heat stress due to the unorganized nest design. At the remainder of the temperatures, WT/WT male mice have consistently poorer nest scores than WT/MUT and MUT/MUT males. At 22°C and 25°C MUT/MUT have the highest nest scores though at 28°C and 31°C this switches to WT/MUT. The general trend suggests that at all temperatures prior to 34°C how males of different genotypes are perceiving the ambient temperatures varies. Mouse strain is known to have an effect on mean nest score suggesting that varying genetics in mice can impact how they perceive heat or simply their nesting behaviors (Gaskill et al., 2013). Considering that besides the mutation, these males are genetically identical, how they perceive temperature due to the mutation appears to be the cause of this variation. Furthermore, PRL has been shown in humans, and arguably cattle through PRLR signaling (Dikmen et al., 2014, 2008; Hammond and Olson, 1994; Olson et al., 2003), to impact how heat is perceived. In a 2006 study, it was found that in humans,

placed in a sauna maintained at 58°C, when face cooling was provided every five minutes, PRL levels showed no difference between the beginning of the trial and after 60 minutes of exposure. On the other hand, if no face cooling was received, PRL levels increased by 102%. Additionally, there was a difference at 60 minutes in the level of thermal discomfort described by the participants with face cooled participants having significantly lower thermal discomfort (Mündel et al., 2006). The manner that WT/MUT and MUT/MUT PRLR signaling is being transduced is presumably different than that of the WT/WT due to the truncation and the removal of two tyrosines, thus potentially changing PRL levels due to PRLR signaling in these mice and causing thermal perception to vary. This however does not explain why there were no differences between genotypes in females for NS. There is not clear physiological explanation for why males would produce a phenotype for nests but females would not and further research should be done to elucidate a potential mechanism. Prolactin has over 300 known functions (Bole-Feysot et al., 1998) and as this list continues to expand, it is possible that a down-stream target in females may not be affected in the same way it is in males causing the difference in the trends observed between genders.

Time at the 28°C to 31°C Temperature Increase's Effect on Nest Score:

Mice have been documented displaying destructive nest behavior due to heat stress by multiple researchers (Gaskill et al., 2012; Gordon, 1993). Their thermoneutral temperature generally resides between 28-30°C depending on the strain and the time of day (Gordon, 2012, 1993). This explains the stark drop in NS in the previous section at the temperature increase between 28-31°C as the mice are apparently transitioning from a thermoneutral to a mildly heat stressed state. Previous studies have also indicated that the

majority of a mouse's time during the light phase is spent being inactive, mostly sleeping (Gaskill et al., 2011). It appears in this study that when the ambient temperature is increased above thermoneutral, in the dark phase, mice will more quickly destroy their nests potentially due to their increased activity during this phase of the day, whereas in the light phase, this process appears to be delayed.

Table 3.1: Sanger DNA sequences from founder male x WT/WT female crosses obtained from the DNA modeling core at the University of Missouri.

Strain	Sanger DNA Sequences	Protein
WT	1 CATCCCTGAGATCACTGAGAAGCCAGAGAATCCTGAGGCAAATATTCCTCCCACCCCAAA 61 TCCCCAAAATAACACCCCCAATTGTCATACAGATACATCCAAATCTACAACATGGCCTTT 121 ACCACCTGGCCAACACACGCGCAGATCTCCTTACCACAGCATTGCCGATGTGTGCAAGCT 181 AGCTGGAAGTCCTGGAGATACACTGGACTCTTTCTTGGACAAAGCAGAGGAAAATGTTCT 241 AAAGTTGTCTGAAGATGCTGGAGAGGAAGAAGTGGCTGTGCAAGAAGGGGCCAAAAGCTT 301 CCCTTCTGACAAACAAAACACATCTTGGCCACCACTCCAGGAGAAAGGCCCCATTGTCTA 361 TGCTAAACCCCCAGATTACGTGGAGATTCACAAAGTCAACAAAGACGGAGTGCTATCATT 421 ACTCCCCAAGCAGAGAGAAAACCACCAGACAGAAAACCCTGGGGTTCCTGAAACCAGTAA 481 GGAGTATGCCA	1 IPEITEKPENPEANIPPTPNPQNNTPNCHTDTSKSTTWPLPPGQHTRRSPYHSIA 56 DVCKLAGSPGDTLDSFLDKAEENVLKLSEDAGEEEVAVQEGAKSFPSDKQNTSWP 111 PLQEKGPIVYAKPPDYVEIHKVNKDGVLSLLPKQRENHQTENPGVPETSKEYA
Line 003	1 CATCCCTGAGATCACTGAGAAGCCAGAGAATCCTGAGGCAAATATTCCTCCCACCCCAAA 61 TCCCCAAAATAACACCCCCAATTGTCATACAGATACATCCAAATCTACA**G**CATGGCCTTT 121 ACCACCTGGCCAACACACGCGCAGATCTCCTTAC**T**ACAGCATTGCCGATGTGTGCAAGCT 181 AGCTGGAAGTCCTGGAGATACACTGGACTCTTTCTTGGACAAAGCAGAGGAAAATGTTCT 241 AAAGTTGTCT**T**AAGATGCTGGAGAGGAAGAAGTGGCTGTGCAAGAAGGGGCCAAAAGCTT 301 CCCTTCTGACAAACAGAACACATCTTGGCCACCACTCCAGGAGAAAGGCCCCATTGTCTA 361 TGCTAAACCCCCAGATTACGTGGAGATTCACAAAGTCAACAAAGACGGAGTGCTATCATT 421 ACTCCCCAAGCAGAGGGAAAACCACCAGACAGAAAACCCTGGGGTTCCTGAAACCAGTAA 481 GGAGTATGCCA	1 IPEITEKPENPEANIPPTPNPQNNTPNCHTDTSKST**A**WPLPPGQHTRRSPY**Y**SIA 56 DVCKLAGSPGDTLDSFLDKAEENVLKLS*
Line 259	1 CATCCCTGAGATCACTGAGAAGCCAGAGAATCCTGAGGCAAATATTCCTCCCACCCCAAA 61 TCCCCAAAATAACACCCCCAATTGTCATACAGATACATCCAAATCTACAACATGGCCTTT 121 ACCACCTGGCCAACACACGCGCAGA**A**CTCCTTACCACAGCATTGCCGATGTGTGCAAGCT 181 AGCTGGAAGTCCTGGAGATACACTGGACTCTTTCTTGGACAAAGCAGAGGAAAATGTTCT 241 AAAGTTGTCT**T**AAGATGCTGGAGAGGAAGAAGTGGCTGTGCAAGAAGGGGCCAAAAGCTT 301 CCCTTCTGACAAACAAAACACATCTTGGCCACCACTCCAGGAGAAAGGCCCCATTGTCTA 361 TGCTAAACCCCCAGATTACGTGGAGATTCACAAAGTCAACAAAGACGGAGTGCTATCATT 421 ACTCCCCAAGCAGAGAGAAAACCACCAGACAGAAAACCCTGGGGTTCCTGAAACCAGTAA 481 GGAGTATGCCA	1 IPEITEKPENPEANIPPTPNPQNNTPNCHTDTSKSTTWPLPPGQHTRR**T**PYHSIA 56 DVCKLAGSPGDTLDSFLDKAEENVLKLS*

Item	FD		WD		W/F		TT		NS	
	F Value	**P-Value**	**F Value**	**P-Value**	**F Value**	**P-Value**	**F Value**	**P-Value**	**F Value**	**P-Value**
Sex	0.46	0.5011	12.52	0.0006	13.67	0.0003	6.32	0.0133	0.04	0.8514
Genotype	1.41	0.2481	1.25	0.2894	1.32	0.2709	4.22	0.0169	4.98	0.0094
Temperature	463.90	<0.0001	59.37	<0.0001	79.64	<0.0001	3264.14	<0.0001	839.94	<0.0001
Sex by Genotype	5.76	0.0041	2.61	0.0776	0.85	0.4305	0.20	0.8182	3.67	0.0301
Sex by Temperature	8.52	<0.0001	16.91	<0.0001	5.26	0.0006	0.52	0.7052	8.43	<0.0001
Genotype by Temperature	4.22	0.0002	1.61	0.1283	2.61	0.0114	0.64	0.7407	2.08	0.0482
Sex by Genotype by Temperature	1.07	0.3899	0.37	0.5770	0.99	0.4491	0.67	0.7131	2.98	0.0060

Table 3.2: Main effects and interactions for all thermal response variables.
Underlined values show significance (P<0.05).

Table 3.3: Main effects of sex, genotype, and room temperature on tail temperature.

Item	n	Tail Temperature (°C) LSMEANS ± SE	P-Value
Sex			0.0133
F	61	28.84 ± 0.05 [a]	
M	65	29.07 ± 0.06 [b]	
Genotype			0.0169
WT/WT	45	28.98 ± 0.05 [a, b]	
WT/MUT	63	29.07 ± 0.04 [a]	
MUT/MUT	18	28.81 ± 0.08 [b]	
Room Temperature			<0.0001
22°C	126	24.14 ± 0.06 [a]	
25°C	126	26.11 ± 0.07 [b]	
28°C	126	29.39 ± 0.05 [c]	
31°C	126	31.84 ± 0.06 [d]	
34°C	126	33.30 ± 0.13 [e]	

Values without a common letter differ (P<0.05).

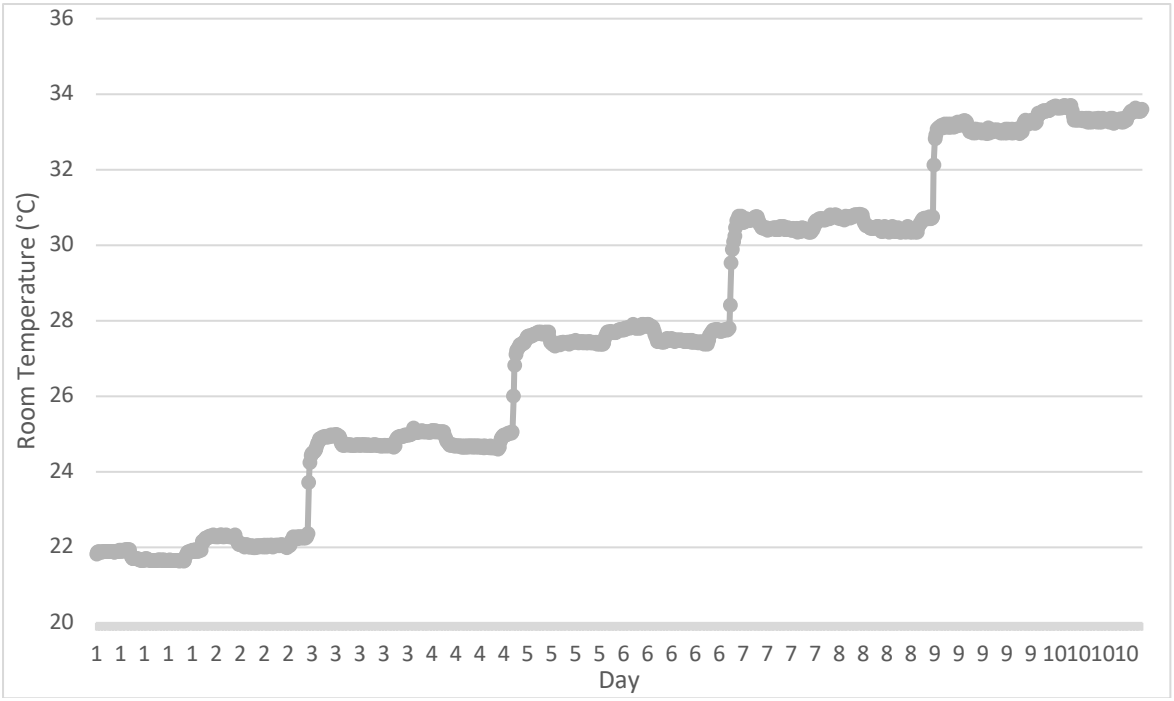


Figure 3.1: Ambient temperature during heat stress trial in Unit B Environmental Chamber. Environmental chambers were programmed to increase 3°C every other day for 10 days. Temperature data was recorded every 15 minutes using Pro V2 Hobologgers ® for the duration of the trial.

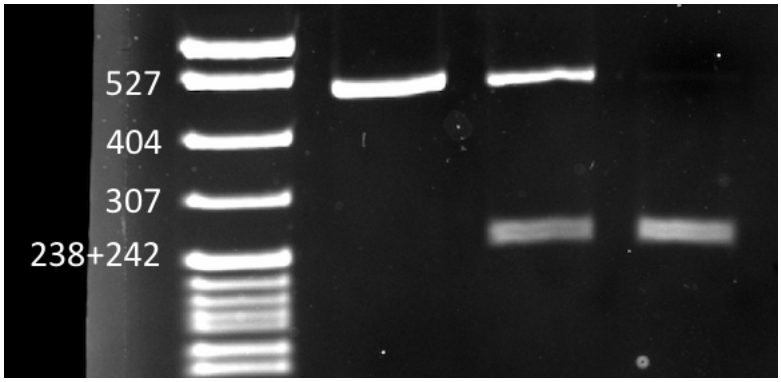


Figure 3.2: PCR verification of truncated PRLR mouse tail snips.
Confirmation of mutation's presence in PCR samples. Lane one contains the pBR322 DNA-MspI ladder. Lane two contains a WT/WT band running at approximately 491 bp. Lane three shows a WT/MUT with one WT, 491 bp, band and a doublet containing a 242 bp band and a 249 bp band indicating a cut, mutant allele. Lastly, lane four contains a MUT/MUT only showing the doublet containing a 242 bp band and a 249 bp band.

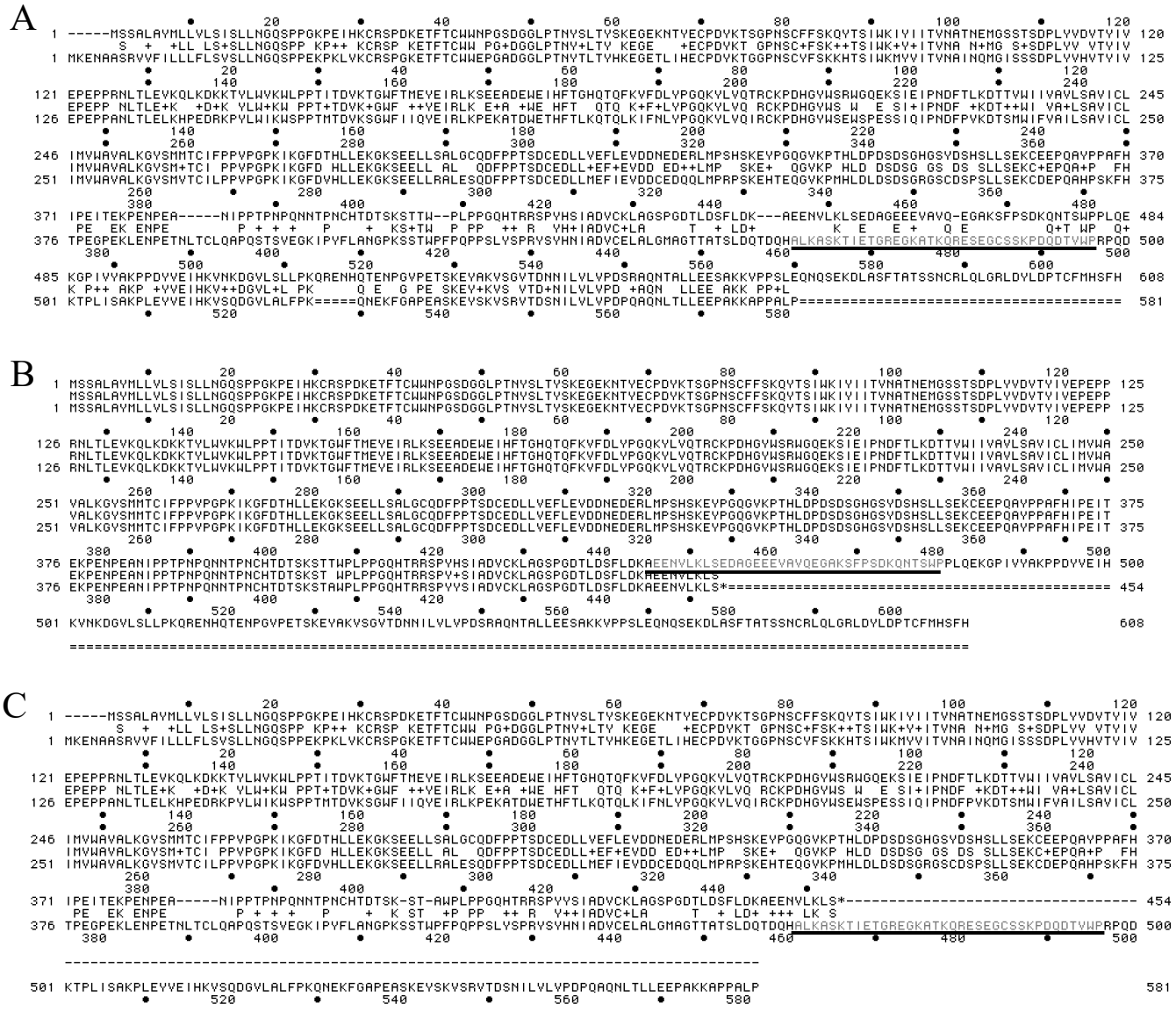


Figure 3.3: Confirmation that line 003's mutation is located within the *slick* region. (A) Upper amino acid sequence is WT/WT C57Bl/6 mouse PRLR. Lower amino acid sequence is WT bovine PRLR with the underlined region being the region where the slick mutations occur. The corresponding segment directly above this region on the mouse sequence was targeted for editing. (B) Upper amino acid sequence is WT/WT C57Bl/6 mouse with the underlined region representing the corresponding region in cattle being the *slick* region. Lower amino acid sequence shows line 003 with a premature stop codon in the desired *slick* region. (C) Upper amino acid sequence shows line 003 with a premature stop codon in the *slick* region. Lower amino acid sequence is WT bovine PRLR sequence. This confirms that line 003 contains a premature stop codon mutation in the underlined, *slick* region.

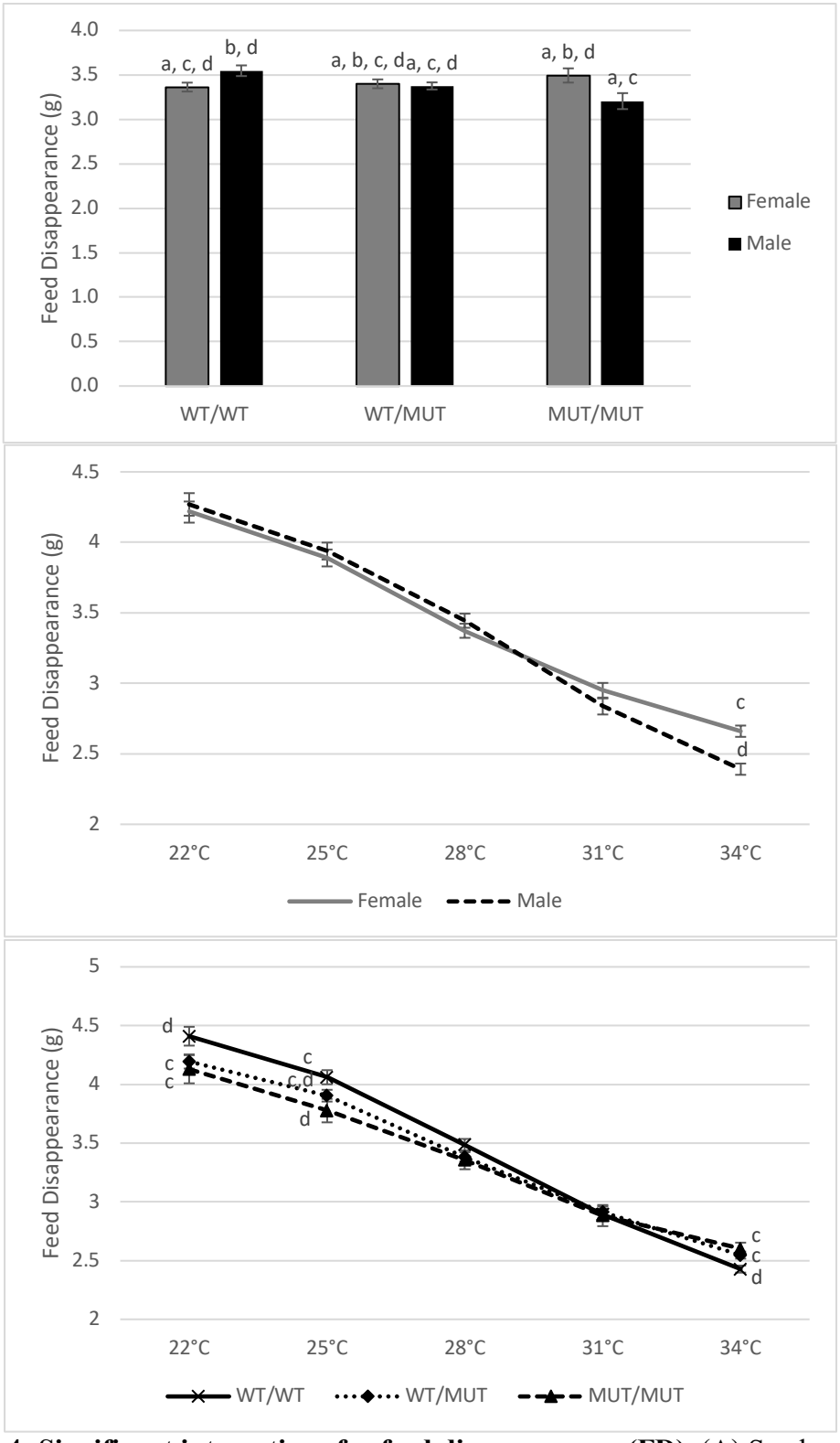
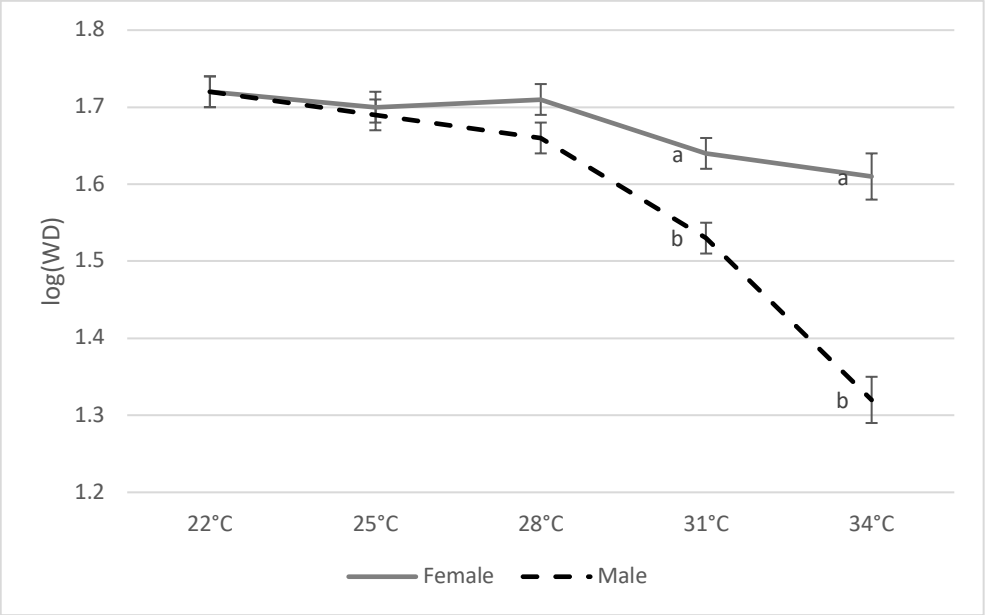


Figure 3.4: Significant interactions for feed disappearance (FD). (A) Sex by genotype interaction. (B) Sex by temperature interaction. (C) Genotype by temperature interaction.
[a, b] Columns a common letter differ (P<0.05).
[c, d] Points within temperature without a common letter differ (P<0.05).

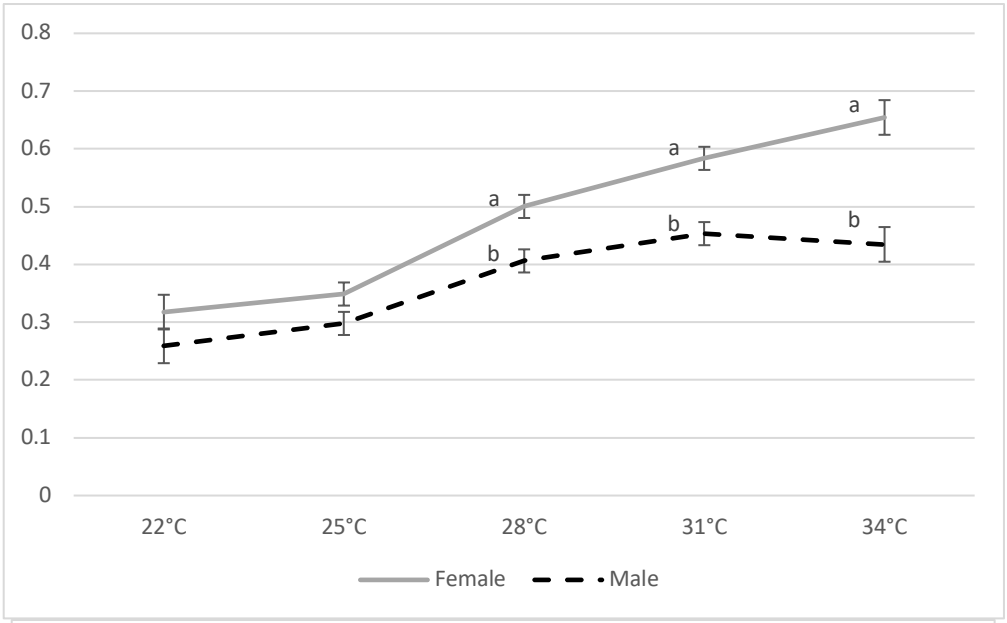
1123



1124
1125

1126 **Figure 3.5: Significant sex by temperature interaction for water disappearance**
1127 **(WD).**
1128 [a, b] Points within temperature without a common letter differ (P<0.05).

A



B

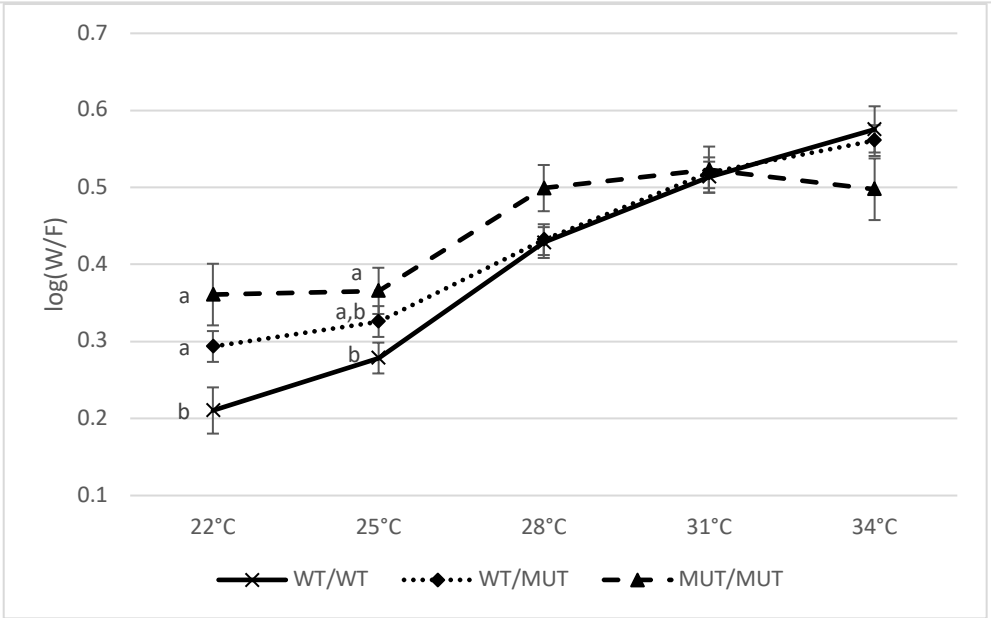
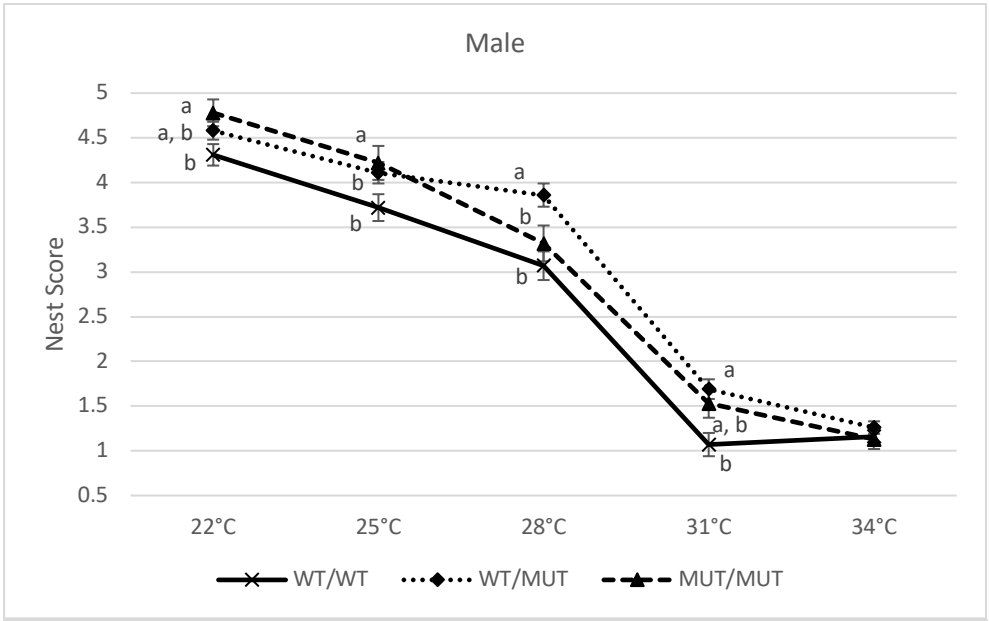


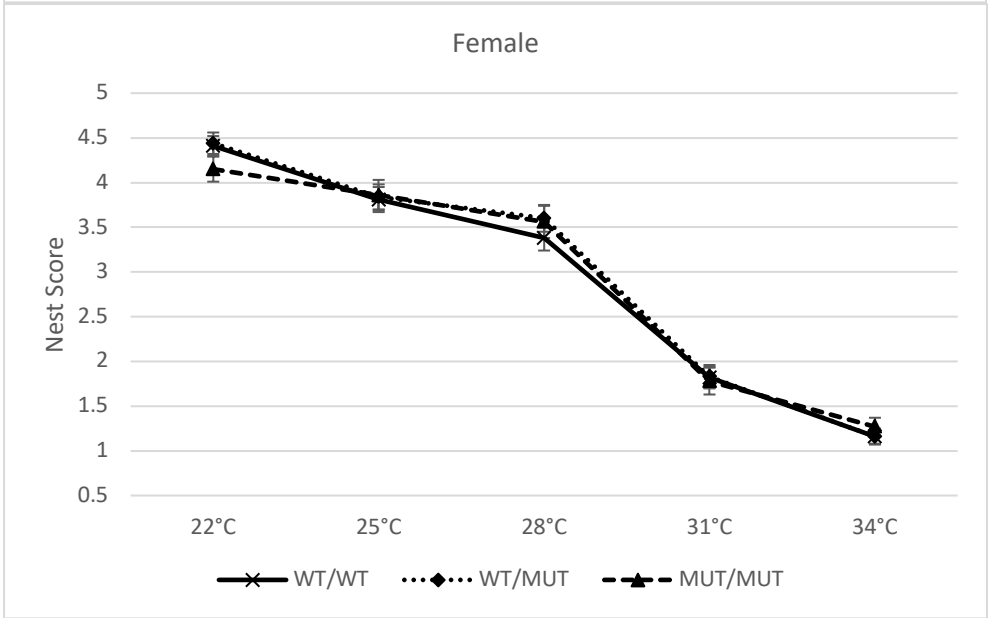
Figure 3.6: Significant interactions for water disappearance per unit feed disappearance (W/F). (A) Sex by temperature. (B) Genotype by temperature interaction. [a, b] Points within temperature without a common letter differ (P<0.05). Note: Data are presented in their original form not the log function form although when processed in SAS, the log function was used when determining significance.

A



1137

B



1138

1139 **Figure 3.7: Sex by genotype by temperature interaction for nest score.** (A) Male
1140 genotypes compared at each temperature. (B) Female genotypes compared at each
1141 temperature.
1142 [a, b] Points within temperature without a common letter differ (P<0.05).

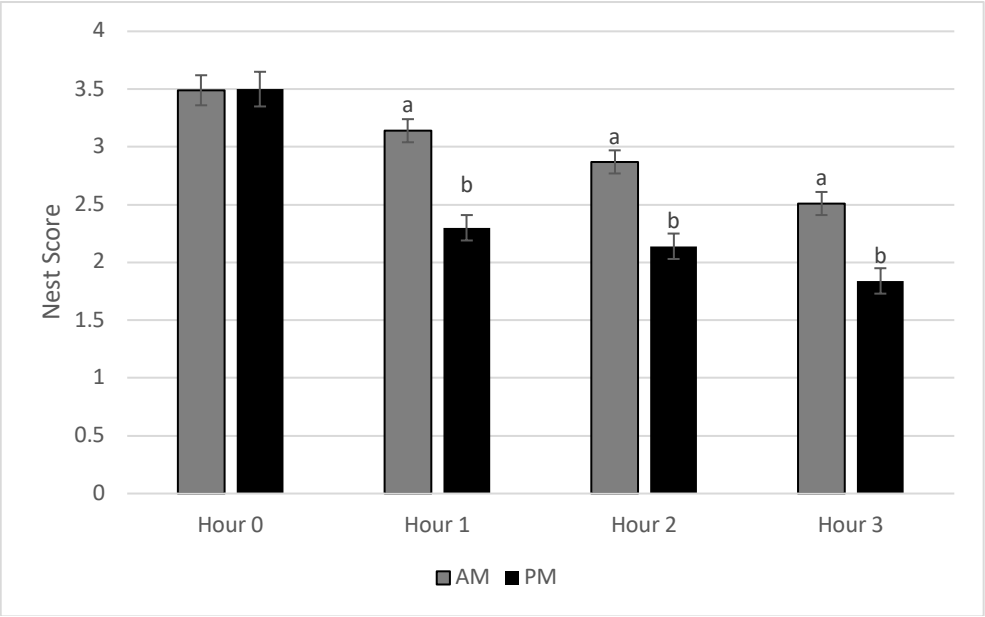


Figure 3.8: Nest scores by hour during the transition from 28°C to 31°C compared based on time of temperature increase.

[a, b] Bars without a common letter within the same hour differ (P<0.05)

CHAPTER 4

EFFECTS OF THE BOVINE SLICK MUTATION ON HAIR REGROWTH IN MICE SHAVED AT 3 WEEKS OF AGE

INTRODUCTION:

In cattle possessing the slick mutation, one of the most apparent phenotypic distinctions between slick cattle and WT cattle is the shorter, sleeker coat in those possessing at least one of the slick alleles (Littlejohn et al., 2014). This mutation causes a premature stop codon to occur and truncates the long form of the prolactin receptor by 85 to 120 amino acids removing two of the seven tyrosines involved in PRLR signaling (Littlejohn et al., 2014; Porto-Neto et al., 2018). Some of the additional benefits of the mutation, including increased thermotolerance, have been hypothesized to be as a result of the varying hair coat.

The long form of the mouse PRLR has been shown to be present in the inner root sheath and outer root sheath in mouse hair follicles. Additionally, the abundance of the receptor varies with the stage of the hair growth cycle (Foitzik et al., 2003). In mice, when the PRLR is knocked out, the days to new hair growth significantly decreased for both males and females (Craven et al., 2001), suggesting PRLR plays a role in hair growth in mice just as it does in cattle. By measuring hair regrowth, it can be determined if hair cycling is impacted by the truncation if there are phenotypic differences between mice possessing the truncation and WT mice. This would ultimately allow us to conclude

if the region associated with the slick mutation in cattle impacts hair growth in mice as well.

MATERIALS AND METHODS:

Animals and Facilities:

All animal procedures were approved and reviewed by the University of Missouri Animal Care and Use Committee (Protocol #9552). The animals used in this study were C57Bl/6 strain from Jackson Laboratories (Bar Harbor, ME), FVB females from Envigo (Indianapolis, IN), and genetically modified C57Bl/6 founder males from the Animal Modeling Core at the University of Missouri. Founder males were mated to wild type (WT/WT) C57Bl/6 females to produce heterozygous (WT/MUT) offspring. These offspring were then mated to produce homozygous (MUT/MUT) pups. FVB females were mated to founder and WT males to produce pups used for hair regrowth trials. Mice were housed in transparent acrylic cages with ground corn cob bedding and a single nestlet (Ancare, Bellmore, NY) per cage. Breeding males were housed individually, and breeding females were group housed with a maximum of four mice per cage. At three weeks of age, pups were weaned, ear notched for identification, and tail snips (less than 5mm) were taken for genotyping. Following weaning, littermates of the same sex were group housed with a maximum of four mice per cage. Mice were housed at a T_a of 23±1°C and were on a 12:12 L:D photoperiod. Feed (5001 Rodent Diet; Lab Diet®, Brentwood, MO) and water were provided *ad libitum*.

At 22 DOA, pups from the matings described above were shaved using and Andis ProClip® Ion Cordless Trimmer (Sturtevant, WI). A 1.5 cm x 1.5 cm patch was shaved

on the back over the hip region. Daily visual appraisal of hair regrowth was recorded at 0900 ± 1 hour until hair the beginning of the hair shaft was visible across the entire shaved patch.

Genotyping:

Genotyping procedures are as described in Chapter 3.

Statistical Analysis:

Data were analyzed using the general linear models procedure of SAS (PROC GLM). Data included one measurement per mouse per day based on the degree of hair growth. The models included the main effects of genotype (WT/WT, WT/MUT, or MUT/MUT), sex, and the genotype by sex interaction. Data for FVB mothered pups and C57Bl/6 mothered pups were analyzed separately. Data are presented as least squares means ± standard error of the least square mean. Means were considered significant at $P<0.05$ and considered to have a tendency toward significance if $0.05 \leq P \leq 0.10$.

RESULTS:

In both the trials, genotype and the sex by genotype interactions did not significantly impact the days to hair regrowth after shaving ($P>0.05$). However, sex did have a highly significant effect on days to hair regrowth in both the FVB x C57Bl/6 and the C57Bl/6 mice ($P=0.0028$ and $P<0.0001$, respectively). In the FVB x C57Bl/6 mice, after shaving, it took 12.5 ± 0.38 days for females to show regrowth, whereas males took 10.86 ± 0.31 days. In C57Bl/6 mice, females again took longer to show regrowth (13 ± 0.1 days) when compared to males (9.61 ± 0.11 days) (Figure 4.1).

DISCUSSION:

The difference due to sex was anticipated as it has previously been described that male and female mice are sexually dimorphic for hair regrowth and time between molts (Craven et al., 2001). When assessed qualitatively, there were also no phenotypic distinctions between any of the genotypes relative to overall coat appearance, including length and thickness. However, the indifference between genotypes was entirely unexpected because of the stark phenotypic differences in hair coat for in cattle possessing at least one slick allele (Dikmen et al., 2014, 2008). Additionally, in a prior study with PRLR KO mice, both sexually dimorphic hair regrowth and a genotypic effect can be observed. When PRLR is knocked out in female mice, the onset of the second hair follicle growth is at 33 DOA and for WT female mice it occurs at 63 DOA. In males, KO mice begin to have new follicles at 31 DOA as compared to WT who show regrowth at 35 DOA. This KO essentially eliminated the sexual dimorphism seen in WT mice, suggesting that PRLR and PRL have a role in the sexual dimorphism of molting in mice (Craven et al., 2001). In addition to this, the conclusion that PRLR and PRL do influence hair growth can be concluded which draws into question why a phenotype was not observed in the WT/MUT or MUT/MUT mice in the present study. This proposes that although the receptor was truncated, the region influencing hair growth in mice is upstream of the mutation and the region that appears to influence hair growth in cattle is not located in the same position in mice.

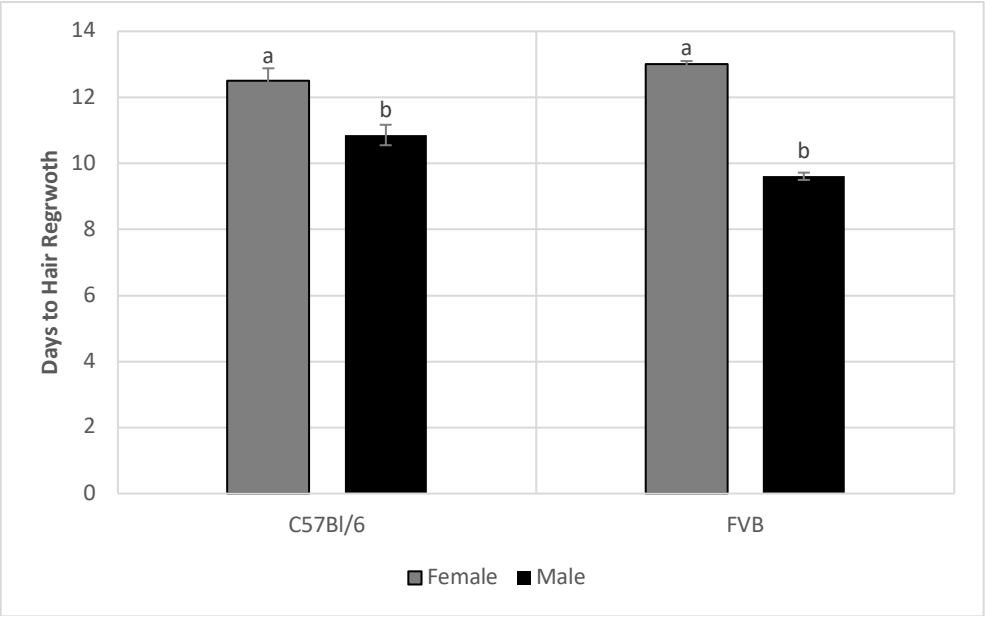


Figure 4.1: Days to hair regrowth compared between sexes in C57Bl/6 and FVB mothered mice.

[a, b] Bars without a common letter within the same genetic background differ (P<0.05).

CHAPTER 5

CONCLUSIONS AND FUTURE RESEARCH

CONCLUSIONS

The bovine slick mutation provides clear improvement during heat stress through a variety of fashions and produces a blatant phenotypic difference in hair coat when compared to WT cattle. When this mutation is introduced to mice, the benefits are not as well defined, nor is there a hair phenotype. Mice possessing at least one mutant allele showed responses that are indicative of improved thermal tolerance for FD and W/F when analyzing the genotype by temperature interactions. MUT/MUT had the highest FD and tended to have the lowest W/F suggesting they are less bothered at the elevated temperatures due to their ability to consume a higher proportion of their baseline feed intake in concurrence with consuming less water per unit of feed than the other mice, indicating they are not using as much water to cool themselves. WT/WT had the opposite results, consuming the lowest FD while producing the largest W/F at 34°C showing that these mice were more bothered as a result of a more severe decrease in feed intake while consuming more water, presumably to aid in heat dissipation. Additionally, a phenotype, not previously described in cattle, was observed in cold stress conditions (22°C and 25°C) with MUT/MUT also appearing to be less bothered by these temperatures due to their lower FD when compared to WT/WT who may be consuming more feed to maintain their core temperature.

This intermediate phenotype was not present when analyzing the TT data. In this data set, MUT/MUT again appeared to have the advantage with a lower average tail temperature; however, WT/MUT and WT/WT tail temperatures were not different from each other and significantly higher than those of the MUT/MUT. Based on TT it appears both copies of the allele are necessary to improve heat tolerance. Nest score also shows less clear results as there were no effects of genotype in the female mice at any temperatures. Males only do not vary at 34°C in which all have nest scores near 1 indicating heat stress. Prior to this WT/MUT and MUT/MUT always have numerically higher nest scores than the WT/WT mice. This could allude to a difference in thermal preference, a behavioral modification, or due to the subjectivity of the measure, variation could be found.

Hair phenotypes previously described in cattle were not observed in mice. Their hair coats did not appear to be "slick," and therefore hair regrowth measures were recorded. Genotype was not found to change days to hair regrowth. Sex was found to elicit an effect, but this result was anticipated based on previous research. This suggests that in cattle, the improvement is not solely due to hair coat density and underlying physiological mechanisms associated the PRLR are the cause. Therefore, although more thermal tolerant, the mutation does not behave the same in both species.

In conclusion, mice possessing the bovine slick mutation do show improved thermal tolerance for those measures generally associated with heat stress studies; however more work needs to be done to determine if core temperature, instead of tail temperature, or other behavioral effectors are altered in the mouse as a result of this mutation. This mutation also appears to be involved in cold stress indicating that this

mutation may be involved in thermoregulation at both extremes of the temperature spectrum, not exclusively heat stress. Furthermore, the slick mutation does not appear to produce a hair coat phenotype in mice although a glaring phenotype is observed in cattle.

FUTURE RESEARCH

In this trial, it was demonstrated that the slick mutation, when introduced into mice, showed improved thermal tolerance in terms of FD and W/F, although no hair phenotype was observed. Moving forward, there is value in determining the magnitude of the effect hair coat has when heat stressing both shaved slick and shaved WT cattle on various measures pertaining to heat stress such as feed disappearance, water disappearance, core temperature, and skin temperature. Due to the novel cold stress phenotype seen in the MUT/MUT mice in this study, there is value in completing trials with slick versus WT cattle in colder environments to account for variation in production that may be seen in winter months. Additionally, the degree of sequence similarity in the *slick* region for cattle versus mice is very low compared to what is observed when paralleling cattle and pigs. Translating this research to a livestock species that commonly suffers from summer heat stress, then conducting heat stress trials in that species, could allow for production of an animal that is more resistant to the elevated temperatures typically observed in the summer months.

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VITA

Shelbi Danielle Perry was born in Lafayette, Indiana on February 26, 1997 to Brian and Christi Perry and was raised in Chalmers, Indiana. Shelbi's participation in youth livestock projects lead her to attended Purdue University where she graduated with a Bachelor of Science degree in Animal Sciences. While there, her involvement in undergraduate research sparked an interest in swine reproductive physiology leading her to the University of Missouri. She will complete a Master of Science degree in Animal Science with an emphasis in swine reproduction and heat stress from the University of Missouri in September 2020, under the guidance of Dr. Timothy Safranski. Shelbi has accepted a potion with Merck Animal Health upon graduation.