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Determining the Relationship Among Cattle Genotype, Hair Coat Score, and Productivity Through the Investigation of Single Nucleotide Polymorphisms within Prolactin, Dopamine Receptor D2, and Melatonin Receptor 1A

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By

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Abstract:

Prolactin (PRL), melatonin (MTN), and dopamine (DA) are all hormones that are believed to play a role in the regulation and growth of hair in beef cattle. There are also single nucleotide polymorphisms associated with each of these hormones or their receptors, indicating that the investigation of these polymorphisms could allow them to serve as genetic markers for the future productivity of an animal. The objective of this study was to determine the relationships among cattle genotype, hair coat score, and productivity through the investigation of single nucleotide polymorphisms within prolactin, dopamine receptor D2, and melatonin receptor 1A. Body weights, hair coat scores, and blood samples were collected in May, June, and July from each non-lactating crossbred beef cow (n=71). The cows were grazing mixed grass pastures that included native endophyte infected tall fescue. Serum PRL, MTN, and DA concentrations were established by validated RIA. Based on the measurements recorded from the May samples, cows were categorized as high (n = 11; 159 ± 29 ng/mL PRL), medium (n = 48; 51 ± 4 ng/mL PRL), or low (n = 12; 21 ± 4 ng/mL PRL). Data were analyzed with Pearson correlations and repeated measures ANOVA with year, month, prolactin category (PRLCAT) and genotype as the main effects. Concentrations of PRL were correlated ($r > 0.53$; $P < 0.0001$) over the three months, and May PRL concentrations were correlated ($r > 0.29$; $P < 0.02$) with cow body weights in May, June, and July. Cows in the low PRLCAT had lower ($P<0.01$) concentrations of PRL in all three months. Hiar coat score decreased ($P < 0.0001$) each month, and was higher ($P <0.05$) for cows with low PRLCAT. Cow body weight increased ($P < 0.0001$) from May to July. Cows in the low PRLCAT had lower ($P < 0.05$) body weights than medium and high PRLCAT cows. These results indicated that concentrations of PRL in May could be useful in identifying cattle with slick hair coats and heavier body weights. Two SNP sites were identified in the PRL gene; A1134T and G8398A. Cow hair coat score was affected ($P < 0.0003$) by an interaction between A1134T and month of data collection. Prolactin coding sequence polymorphism G8398A affected ($P < 0.0001$) cow body weight and hair coat score. Cows that were homozygous for the primary allele (GG) had lower ($P < 0.05$) hair coat scores than those that were homozygous for the minor allele (AA). No correlations were identified with the melatonin or dopamine receptors. These two specific polymorphisms associated with prolactin could be significant predictors in cow performance and productivity

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Chapter 1: Introduction

The beef cattle industry has continued to gain increasing significance world wide as more countries begin to undergo urbanization and as the world's population grows at significant rates. As long as these trends progress, as they are forecasted to, there will continue to be an increased demand for livestock products (Hayes et al., 2013). The beef cattle industry has already proven to be one of the most important and impactful production systems in the World, but it holds significance in the sustainability and agricultural economy of many countries individually as well (Thornton, 2010). The United States is a perfect example of this in that beef is one of the country's largest livestock outputs and according to a report released by NASS (2016), cattle production represented 21 percent of the Economic Research Service's forecasted total cash receipts of $377 billion from agricultural commodities in 2015. Based on the impact the beef industry is having throughout the world, it is essential to optimize this industry in any way possible (Hayes et al., 2013). One clear way to make the most of resources is to insure cattle are being produced at optimal weights and have the highest calving rates as well as weaning weights possible. Those in the cattle industry tend to use the standards of calving rate, calving weight, and weaning weight as a marker for the successfulness of a cow as a whole as well as that cow's individual productivity (Wiltbank, 1994). That being the case, it is desirable to breed and grow cows that have the highest rates in these areas.

Advancements in genetic technology have allowed us to understand on a much deeper level the genetic factors affecting important productivity traits, such as winter hair coat loss (Meuwissen et al., 2001). Researchers have found the shedding of a cow's winter coat to be an incredibly important productivity factor, as the retention of the coat into late summer causes a

significantly higher amount of heat stress on an animal than that seen in animals that shed their coat early in the summer season, and as a consequence of this increased heat stress, animals show a lowered productivity (Porter and Thompson, 1992). Recent discoveries, suggest that there are three crucial physiological markers that are heavily involved in the economic traits of cattle. These are prolactin (PLN) (Craven et al, 2001), melatonin receptor 1a (MTNR1A) (Santiago-Moreno et al., 2004), and dopamine receptor D2 (DRD2) (Campbell et al., 2014). These three variables, and the genetic markers associated with them, are believed to be able to serve as biomarkers for heat stress in animals due to their role in the hair coat score and productivity of angus- based cattle. However, their part in this score can also be directly affected by an animal's exposure to and consumption of endophyte infected tall fescue, which may also cause a decreased hair coat score -- indicating longer retention (Porter and Thompson, 1992).

The study of these markers points to a clear correlation between the differences in nucleotide arrangement and an animal's overall productivity and, specifically, hair coat score. Due to the fact that hair coat score, and consequently the time of year in which an animal sheds their coat is so crucial to the overall productivity and successfulness of the animal, it is important researchers continue to investigate and understand the exact impact such genetic variables have. In the case that scientists are able to fully discern the specific role of these genes on hair coat score and other economic/productivity traits, cattle farmers will be able to selectively breed cattle based on their genotypic characteristics as well as their phenotypic traits (Garrick, 2011). This would allow researchers and farmers to predict a productivity outcome long before they have been able to in the past due to the technology restrictions that were only recently overcome (Schefers and Weigel, 2012). The implications of this are that cattle farmers in the United States and across the world would be able to raise more productive herds than has

ever been feasible in the past, and this could revolutionize the cattle industry and aid in the ever growing demands on it (Hayes et al., 2013).

A study done in 2008 (Coffey et al., 2008), and one done in 2011 (Caldwell et al., 2011) showed that there was a correlation between the dates calves were weaned and their overall weaning weight. In the 2011, study, it was clear that calves weaned later in the year - early June- had higher weaning weights than those weaned earlier- mid April. It is hypothesized that this difference could have occurred in large part due to the fact that concentrations of ergovaline (an ergot alkaloid in E+ fescue plants which is thought to impact the performance of animals in a negative way) see a significant increase between the months of April and June (Rottinghaus et al., 1991). However, the results found in these two studies conducted on fall born calves, differed from the results of a study performed in 1999 on spring born calves (Myers et al., 1999).This study reported the opposite findings of those performed in the fall, such that there was a linear decrease in overall daily gains across weaning weights. The difference in these studies highlights the importance to compare results of studies done across different months and seasons, specifically when endophyte infected tall fescue is an active factor.

A study was conducted by Meyer. L. R (2016) in order to determine the impact of single mononucleotide polymorphism in Prolactin, MR1A, and DRD2 on cow-calf profitability traits, and found that the results were significant enough to conclude that these three biological markers could be used in cattle selection in the future. This study was conducted on calves born in August-December. So, there is still question as to whether these results would vary if the tests were conducted with spring born calves.

Based on this, the objective of this study was to further the investigation of the correlation between prolactin, dopamine receptor D2, and melatonin receptor 1a, and

productivity as well as hair coat scores, so as to describe the most genetically favorable cow for farmers to grow and breed. It specifically focused on the body weight, hair coat scores, and blood serum levels of the three respective physiological markers mentioned (prolactin, melatonin, and dopamine).

Definition of Terms

Productivity: For the purposes of this study, we will be defining a cow's productivity as their overall efficiency in regards to body weight and hair coat score.

Limitations of Study

This study is limited in the number and variance of subjects being tested. We researched Angus based beef cows on a farm in Savoy, AR, however, if other subjects in other states and climates were able to be included in the study that would be preferable.

This study was also limited by time. It is desirable to have results recorded over the course of several years in order to draw strong conclusions, but due to time constraints or study will only cover the span of one year.

Chapter 2: Review of Literature

Hair Characteristics

Hair Growth Cycle

A defining characteristic of mammals is the presence of hair, and it serves a variety of functions. It can provide protection from the elements and parasites/pathogens, aid in thermal insulation, act as camouflage, disperse sweat and sebum, amplify senses and tactile functions, and can even be instrumental in social interactions (Schneider et al., 2009). Each hair follicle will proceed through a cycle of three phases of growth. A mature hair follicle will have two clear divisions which include a permanent upper part, and a lower part which is the section that is undergoing physical change during each cycle. These phases include a phase of growth (anagen), regression regulated by apoptosis (catagen), rest (telogen), and shedding (exogen)(Schneider et al., 2009, Stenn et al., 2001). According to research done by Stenn, 2009, it is probable that this cycle of growth and shedding is mediated by similar cellular signals to the ones essential to other morphological characteristics such as the salivary glands, kidney, breast and teeth.

Thermal Stress

Mammals have several different means by which they regulate body temperature in order to maintain homeostasis. These include conduction, convection, radiation, evaporation of water, and through expiration when breathing. In cooler temperatures, they will dissipate heat via radiation and convection, and in higher temperatures they will switch to vaporization. So, when animals begin to experience thermal stress, in which their bodies are reaching temperatures above what is required for homeostasis, their body responds by reducing water loss through the

expiration of feces and urine, reducing feed intake, and increased sweating. They will also increase rate of respiration and heart rate in their initial response, but these will slow if the stress continues (Kadzere et al., 2002).

Cattle will primarily dissipate heat by evaporative cooling (Gray et al., 2011). This is mainly due to the inherently large mass of cattle, which makes peripheral vasodilation an efficient method of heat dissipation (Kadence et al., 2002). Unfortunately, as the temperature moves beyond an animal's thermal neutral zone, and as humidity increases, evaporative cooling via sweating and respiration becomes much less effective (Gray et al., 2011).

Thermal Stress takes place when an animal's homeostasis is disrupted and their core body temperature rises above what is considered the normal range, and exceeds what can be managed by heat dissipation (Bernabucci et al., 2010). Because of the correlation between the ability of an animal to dissipate heat effectively, and the humidity of the region they live in, cattle in the southeast region of the United States (where there is a subtropical climate) are at a higher risk for heat stress. This means cattle that have darker or thicker coats, and those that shed their coat later in the year, are at a very high risk of experiencing heat stress as well as dehydration (Gray et al., 2011). Heat stress poses a very real threat to cattle farmers, the agricultural economy, and the food supply for humans, because of its negative impact on the productivity of an animal (Bernabucci et al., 2010). The decrease in productivity due to heat stress is primarily due to the fact that animals will respond by reducing dry matter intake, as ruminant fermentation and metabolic functions generate heat. (Beede et al., 1986)

Fescue Toxicosis

Tall fescue (*Festuca arundinace*a) is known to cause fescue toxicosis in cattle that graze it (Stuedemann and Hoveland, 1988). It is thought that the alkaloids produced by the endophytic fungus (*Neotyphodium coenophialum*) are what cause the symptoms of fescue toxicosis (Hill et al., 1994). The endophyte is present in the plant as a way to increase its overall persistence, making it more durable than tall fescue absent of the endophyte (Clay, 1988). In a study done in 1996, both steers and lambs that grazed endophyte-infected tall fescue experienced decreased blood flow. In lambs the decreased blood flow was exhibited in the leg skin and adrenal glands, and in steers it was seen in the rib skin, cerebellum, duodenum, and colon. The results of this experiment indicated that the peripheral organs and limbs, the core of the body, and the areas of the brain responsible for temperature regulation are what see the effects of the fescue. This decrease in blood flow could be a direct result of the inability to dissipate body heat (Ben-Jonathan, 1996).

Genetic Characteristics

Genomic Technology

There are several characteristics in beef cattle that are important in terms of productivity, and economic value, including carcass characteristics and growth, that may not be easily measurable pre-harvest. In these cases, finding links between specific genetic markers and these traits, can greatly improve the quality of cattle being raised, and can do so prior to the investing time and resources into genetically unfavorable animals (Thompson et al., 2014) This makes genetic testing an increasingly valuable tool in selection decisions within the beef cattle industry (Van Eenennaam et al., 2011, Thompson et al., 2014, Wimmer et al., 2013). The cost of this genomic

technology has decreased significantly in recent years, while also improving in accuracy. This type of testing can provide producers with genetic information pertaining to genetic defects, genetic markers, and even single nucleotide polymorphisms (SNP), which can give insight to qualitative traits and quantitative traits at an earlier age than would be possible without this technology (Thompson et al., 2014, Shefers and Weigel, 2012).

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNP) are the result of altered nucleotides [adenine (A), thymine (T), cytosine (C), or guanine (G)] in the genetic sequence of an animal (Looper et al., 2010). There are now methods readily available to the general public, utilizing SNP based genotyping. These tests can use SNP ranges in the genome of individual cows to explain genetic variations in productivity traits between them (Shefers and Weigel, 2012). As explained by the work done by Shefers and Weigel, 2012, this technology utilizes prediction equations established from a reference population of animals that display the traits breeders deem desirable, and can do so accurately even before the animals have reached sexual maturity.

Prolactin

Prolactin Physiology

Prolactin (PRL) is a protein hormone secreted from the anterior pituitary gland by lactotropic cells (Looper et al., 2010, Riddle et al., 1933). The secretion of prolactin is regulated via inhibition by dopamine (Paterson et al., 1995). Prolactin plays a role in many more physiological functions, such as regulation of mammary gland development, promotion of lactation, osmoregulation, and behavioral changes, than any other hormone secreted by the pituitary gland

(Ben-Jonathan, 1996). Prolactin also plays a role in many endocrine functions related to reproduction and lactation in mammals, and does so through a JAK/STAT signal transduction pathway (Bole-Feysot et al., 1998). Its role in mammary gland development and control of lactation in the end phases of pregnancy are considered the primary role of prolactin (Riddle et al., 1931). Prolactin is also believed to inhibit postpartum ovarian activity due to suckling (Wheeler et al., 1982). This wide range of physiological action demonstrated in prolactin can be acquainted with three components of the hormone -- structural polymorphism, local production and processing, and the wide range of intracellular signaling pathways as well as target genes (Ben-Jonathan, 1996).

Prolactin Receptor (PRLR)

The many physiological roles of prolactin are regulated by its receptor, prolactin receptor (PRLR), an anterior pituitary peptide hormone (Bole-Feysot et al., 1998), which can be classified with the cytokine receptor family based on its ability to activate the JAK/STAT pathway (Fleenor et al., 2006). Several studies conducted recently, have indicated the association between nucleotide polymorphisms within the PRLR gene and swine reproductive traits (Barreras et al., 2009, Tomas et al., 2006, Kmieć et al., 2006, and Rens et al., 2003).

Dopamine

Dopamine Physiology

Dopamine (DA) is the catecholamine neurotransmitter that predominates in the mammalian brain. It is responsible for the regulation of many functions such as locomotion, cognition, emotion, reinforcement, feed intake, and endocrine regulation (Misale et al., 1998). Catecholamines function by sending signals between neurons and act via synapses (Ben-Jonathan and Hnasko, 2001). Within the periphery, DA functions as a regulator of cardiovascular function, the release of other catecholamines, hormone secretion, tone of vasculature, renal function, and gastrointestinal motility (Misale et al., 1998). The central nervous system is the primary site of synthesis of DA, but some production also takes place in the adrenal medulla. (Ben-Jonathan and Hnasko, 2001). An important structural factor of DA is that it is classified as a monoamine, which means it is a small, water-soluble, derivative of an amino acid, and that it can be found in secretory granules in high concentrations. The significance of this being that the granules protect DA from degradation by metabolic enzymes, and allow for regulated release through exocytosis (Ben-Jonathan and Hnasko, 2001).

Dopamine Receptors

The signaling of Dopamine is conveyed primarily via the action of two receptors, D1 and D2 (Kebabian and Calne, 1979, Dal Toso et al., 1989) As of right now, scientists have identified five unique receptors, but these are classified by two different subfamilies; D1-like family (D1 and D5) and D2-like family (D2, D3, and D4). All of these receptors can be classified as G-protein coupled receptors, and are made of single polypeptide chains that can vary in size from 387-475 residues. Dopamine Receptor D2 (DRD2) is the receptor responsible for the regulatory

action of dopamine on prolactin (Ben-Jonathan and Hnasko, 2001) There are several signaling events that occur at the D2 receptor. Changes in K+ (Israel et al., 1988, Castelletti et al., 1989) and Ca2+ (Tarakevich and Douglas, 1978) ion channel activity are an example of these events. Some phosphoinositol hydrolysis-coupled changes in the calcium concentration also occur at this receptor (Beaudry et al., 1986, Enjalbert et al., 1986). D2 receptor stimulation is also known to reduce the activity of adenylyl cyclase (Dal Toso et al., 1989).

Dysfunctions of Dopamine

There are several diseases linked to dysfunctions in dopamine. These include Parkinson's disease, which is caused by a dopamine deficiency in the midbrain. Inversely, an over activity of dopaminergic neurons within the limbic and cortical systems has been linked to schizophrenia and psychoses (Ben-Jonathan and Hnasko, 2001). Another defect that can arise in association with dopamine is a lactotroph insensitivity to dopamine within the pituitary, which can lead to hyperprolactinemia and infertility (Dal Toso et al., 1989, Ben-Jonathan and Hnasko, 2001).

There are also significant health consequences related to the dopamine receptors themselves; D2 in particular (Ben-Jonathan and Hnasko, 2001). For example, the aforementioned link between dopamine and schizophrenia, is thought to be a result of a malfunctioning of D2 receptor-mediated signaling between the midbrain and lambic/cortisol regions (Dal Toso et al., 1989). Dopamine, similarly to prolactin, can be affected by the ergot alkaloid component of tall fescue. These alkaloids bind to DRD2, which affects the density of the receptor (Larson et al., 1999). Because of the relationship between DRD2 and the release of prolactin (Lamberts and Macleod, 1990), the inhibition of the release of prolactin (Strickland et al., 1992, 1994), can be used as an

indicator to whether or not livestock is consuming tall fescue (Stuedemann and Thompson, 1993). Based on its diverse activity within the body, it is believed that DRD2 is mediated by at least two different G proteins -- Gi and Go, and that the structural heterogeneity of the receptor allows it access to the intricate anatomy of the target cells of dopamine. For this reason, drugs designed to alter dopamine activity typically target the D2 Receptor specifically (Dal Toso et al., 1989).

Melatonin

Melatonin Physiology

In mammals, circulating melatonin (MTN) is primarily secreted by the pineal gland (Zagajewski et al., 2012, Bubenik, 2002). The gastrointestinal tract, epithelial hair follicles, skin, retina, salivary glands, platelets, lymphocytes, and the brain while it is in the developmental stages, are all also able to produce melatonin (Izykowska et al., 2009, Yoshida, 2013). By inhibiting cGMP and cAMP, melatonin regulates gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) (Sampaio et al., 2012). Melatonin reaches very high levels within ovarian follicular fluid, where it can be synthesized, and is more concentrated here than within in the plasma (Sampaio et al., 2012). It is believed that the synthesis of serotonin via granulosa cells may be used in the synthesis of ovarian melatonin. Melatonin is mainly synthesized by parenchymatous cells within the pineal gland in response to input from the light (Singh and Jadhav, 2014). There are serious health risks associated with melatonin shortages within the body such as Parkinson's disease, insomnia, epilepsy, ischemic injury and neuropsychiatric disorders. It is also believed that melatonin is linked directly to eye function in

that it may play a role in retinitis (inflammation of the retina) and cataract development (Shimozuma et al., 2011, Wehr et al., 2001).

Melatonin plays an important role in the growth and maturation of oocytes, specifically by its action as a calmodulin and antagonist and radical scavenger in oocytes (Ishizuka et al., 2000, Del et al., 2004, Sakaguchi et al., 2013). Melatonin has been implicated as a key component in the reproduction of mammals (Srinivasan et al., 2009), and specifically in seasonal gonadal activity (Singh and Jadhav, 2014). In two recent studies, the administration of melatonin over a 1 hour period in adult male Siberian hamsters, showed an altered functional status of the testes (Amireualt et al., 2005, Turk et al., 2003).

Melatonin also has lasting effects on embryos, specifically in cellular cleavage and in the rate of formation of the blastocyst (Sampaio et al., 2012, Singh and Jadhav, 2014). Studies done in mice (McElhinny et al., 1996, Ishizuka et al., 2000), pigs (Rodriguez et al., 2007), and buffalo (Manjunatha et al., 2009) showed a correlation between the production of fertilized embryos and the concentration of melatonin within the oocyte.

Melatonin Receptors

Melatonin's membrane receptors are responsible for some of the effects from melatonin within the ovary, and other reproductivity related functions (Romero et al., 1998, Malraux et al., 2001, Prendergast, 2010, Trecherel et al., 2010, Carcangiu et al., 2009, 2011). There are two subtypes of melatonin receptors, MT1 and MT2, each of which are G protein-coupled transmembrane receptors, and can be found within matured ovaries. It is by MT2 that melatonin limits the

production of cGMP and inhibits granules cyclase (Singh and Jadhav, 2014). The inhibiting action of melatonin on adenylate cycle as early enzyme is also through MT1 and MT2 (Slominski et al., 2008, Dubocovich, 2005). MTQ is also responsible for the activation of phospholipids, which is the means of ion flux regulation within the cell (Singh and Jadhav, 2014).

Melatonin and the Circadian Rhythm

In seasonally breeding animals, melatonin and dopamine antagonists have been shown to influence gonadotroph and seminiferous tubule size (Singh and Jadhav, 2014). In any living organism, environmental lighting is responsible for temporal changes, and in mammals the major site of this biological time regulation is the suprachiasmatic nucleus (SCN). The circadian rhythm includes both a dark and light phase, and indole amine melatonin is released due to signals by the SCN to the pineal gland during the dark phase (Sampaio et al., 2012). Because melatonin secretion is occurring primarily during the dark phase, it acts an indicator of the photoperiodic trend (Lincoln and Hazlerigg, 2010). Because it is coupled with the "biological clock" there are clear indications of seasonal variation of reproductive activity in small ruminants via melatonin (Bartness et al., 1993). Melatonin even signals day length to a developing fetus, thus regulating the development, by crossing the placenta (Irmak, Topal, and Oter, 2005, Torres-Farfan et al., 2006, Dubocovich, 2007, Seron-Ferre et al., 2007). These circadian relations of melatonin are regulated by its receptors within the SCN (Weaver et al., 1996) and the reproductive effects occur and are regulated within the hypothalamus (Migaud et al., 2005)

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Chapter 3: Methodology

The objectives of this study were to:

- collect blood samples from participating cattle
- Centrifuge and collect Buffy coats for testing
- Quantify DNA using Qubit ® Fluorometer
- Analyze genotype data provided by Geneseek, using the ANCOVA model
    - They used the sequenom technique
- Determine the relationship between the single nucleotide polymorphisms within prolactin, dopamine, and melatonin, and hair coat score/productivity

Participation and Sampling

For this study, we utilized samples from nonlactating crossbred beef cattle (n=71) on a farm in Savoy, AR. The body weight and hair coat score was reported and a blood sample was collected for each of the cows during the months of May, June, and July. The blood samples collected at this time were used to determine the prolactin, melatonin, and dopamine levels for each cow during each month. These results were then used to test the correlation between the hormone levels of each cow and their corresponding monthly hair coat score. Their genotype was reported using the services provided by Geneseek (Geeneseek, Lincoln, NE) and compared with their hair coat score and hormone levels in order to determine the correlation between these factors.

Treatments and Instruments

Treatments:

Because this study used a non-experimental correlational design in order to determine the relationship between cattle genotype, productivity, and hair coat score, no treatments were applied. The conditions of all cattle being studied were the same. All cattle participating in the study were grazing mixed pastures of bermudagrass and endophyte infected tall fescue, and had ad libitum access to trace mineral supplements.

Instruments:

- EDTA treated tubes: EDTA stands for ethylenediamine tetraacetic acid, which is an anticoagulant that is commonly used to prevent clot formation in blood. This is why we used test tubes treated with EDTA to collect blood samples from all participating cattle.

- Centrifuge - this instrument was used to isolate the Buffy coat from blood sample, which is the component of the blood that contains the DNA information.

- DNeasy blood and tissue kit - This instrument allowed us to extract the DNA from the Buffy coat, and purify it from the animal's blood and tissues and from the cells, yeast, bacteria, or viruses. The kit is a standardized method for many different sample types, and provides high yields of high quality DNA.

- Qubit ® Fluorometer - this instrument, which uses the different wavelength distribution of the emission spectrum after excitation by a certain spectrum of light to detect the

presence and number of molecules in a particular substance, was used to quantify the DNA.

- Well plates and drying oven - This instrument was used to prepare DNA to be shipped to Geneseek in Lincoln, NE. The DNA had to be dried and properly sealed within the well plates in order to be shipped to Geneseek and analyzed. The results from Geneseek was used to determine which single nucleotide polymorphisms were associated with hair coat score and the levels of the physiological markers being studied.

Data Collection

We began collecting blood samples in May using EDTA treated tubes, and then carried out the genotyping process. This entailed the use of the centrifuge to isolate the buffy coat from the blood sample, which is the component of the blood that contains the DNA information. Then, by using the DNeasy kit, we extracted the DNA from the buffy coat, and subsequently purified it from the animal's blood and tissues and from the cells, yeast, bacteria, or viruses. Following these steps we shipped the serum samples to a test lab at Louisiana State University (Baton Rouge, LA), where they used electro immunoassay to quantify the levels of each of the three hormones being studied and reported the results back to us. We then used the Quibit © Fluorometer to quantify the DNA, and used well plates and a drying oven to prepare DNA to be shipped to Geneseek in Lincoln, NE. The DNA had to be dried and properly sealed within the well plates in order to be shipped to Geneseek and analyzed. The results from Geneseek were what we used to determine which single nucleotide polymorphisms are associated with hair coat score and productivity.

Data Analysis

Our data was analyzed using mixed model ANOVA with the main effects of year, month, and genotype. The overall means for the hair coat scores over a 3 month period were recorded. We ran a repeated measure analysis using the maximum likelihood method and, the dependent variable was hair coat score. When F-tests for the main effects were reported to be significant ($P < 0.05$), multiple T-tests and the Tukey's adjustment were performed to separate the means.

Using the culmination of these tests, we determined the relationship between cattle genotype, hair coat score, and productivity traits. These findings have been reported along with the relationships that have been determined.

Results and Discussion:

Prolactin:

Following the collection of prolactin samples in May, three categories were established based on the amounts of circulating prolactin measured within each cow. Cows with 159 ± 29 ng/mL PRL were placed in the "high" category (n=11), those with 51 ± 4 ng/mL PRL were placed in the "medium" category (n=48), and individuals with 21 ± 4 ng/mL were categorized as "low" (n=12). These categories were correlated with the three months of study, and specifically the May prolactin levels correlated with the body weights of the cows across all three months. While hair coat score consistently decreased each month, cows within the "low" prolactin category displayed a higher hair coat score throughout. The cows categorized with low prolactin subsequently had lower body weights than those in both the medium and high categories. The

conclusion drawn from these results was that circulating prolactin levels in May could be a key predictor to future body weight.

The results of the SAS output did not show any correlation between coding sequence polymorphisms in prolactin coding sequence G8398A, prolactin promotor polymorphism A1134T, or prolactin promoter polymorphism C1286T and circulating serum prolactin concentrations. There was, however, an effect on body weight noted in polymorphisms associated with prolactin coding sequence G8398A, and an effect on hair coat scores noted in both prolactin coding sequence polymorphism G8398A and prolactin promoter polymorphism A1134T..

A1134T:

Prolactin promoter polymorphism A1134T did not affect ($P > 0.3$) serum prolactin concentrations or cow body weight. However, cow hair coat score was affected ($P < 0.0003$) by an interaction between A1134T and month of data collection. (See Table 6)

G8398A:

Prolactin coding sequence polymorphism G8398A affected (P < 0.0001) cow body weight. Cows that were heterozygous weighed more than cows that were homozygous for both the major and minor allele, but none of the genotypes were significantly different from one another (546, 620, and 541 kg; respectively for genotypes GG, GA, and AA). (See Table 7)

In regards to hair coat score, it was found that cows that were homozygous for the primary allele (GG) had lower ($P < 0.05$) hair coat scores than those that were homozygous for the minor allele

(AA), and the heterozygous cows had the highest hair coat scores, but showed no significant difference from the homozygous cows (2.5, 3.3, and 2.9; respectively for genotypes GG, GA, and AA). (See Table 8)

Melatonin:

Neither of the two melatonin receptor polymorphisms tested, G497A ($P > 0.1$) and A455G ($P > 0.6$) were shown to affect serum prolactin concentrations, hair coat score, or cow body weight

Dopamine:

The dopamine receptor polymorphism A534G showed no affect ($P > 0.1$) on serum prolactin concentrations, hair coat score, or cow body weight.

Tables:

	Prolactin		
	May	June	July
May	50.4 ng/mL	76.8 ng/mL	61.4 ng/mL
June	-	0.56**	0.6**
July	-	-	0.53**

Table 1: correlations between prolactin levels and the months of May, June, and July
*P value less than 0.05
** P value less than 0.01

	Prolactin		
Cow Body Weight	May 50.4 ng/mL	June 76.8 ng/mL	July 61.4 ng/mL
May, 519 kg	0.3*	0.19	0.09
June, 547 kg	0.33**	0.25	0.15
July, 574 kg	0.34**	0.25	0.14

Table 2: correlations between circulating prolactin levels and cow body weight during the months of May, June, and July
*P value less than 0.05
** P value less than 0.01

	Prolactin		
Cow Hair Coat Score	May 50.4 ng/mL	June 76.8 ng/mL	July 61.4 ng/mL
May, 4.2	-0.14	-0.11	-0.02
June, 2.7	-0.19	-0.23*	-0.09
July, 1.3	-0.21	-0.23*	-0.26*

Table 3: correlations between circulating prolactin levels and cow hair coat score during the months of May, June, and July
*P value less than 0.05
** P value less than 0.01

	Sample Month		
	May	June	July
Prolactin, ng/mL	78.6	81.2	71.0
Cow Body Weight, kg	510[c]	539[b]	565[a]
Hair Coat Score	4.3[c]	2.7[b]	1.3[a]

Table 4: variance among the months of May, June, and July, and prolactin, cow body weight, and hair coat score

	Prolactin Category		
	Low n=12; 21 ng/ML	Medium n=48; 51ng/mL	High n=11; 159ng/mL
Prolactin, ng/mL	20.8[c]	51.4[b]	158.5[a]
Cow Body Weight, kg	484[b]	555[a]	575[a]
Hair Coat Score	3.3[b]	2.7[a]	2.4[a]

Table 5: variance among the prolactin level categories (low, medium, high) and circulating prolactin, cow body weight, and hair coat score

	A1134T Genotype		
	AA	AT	TT
May	4.2[a]	4.4[a]	3.0[b]
June	2.7[cb]	2.8[cb]	1.7[cd]
July	1.2[d]	1.4[d]	1.3[d]

Table 6: relationship between cow hair coat score and the interaction between A1134T and month of data collection [[abcd] Least-squared means without a common superscript differ ($P < 0.05$)]

	G8398A Genotype		
	GG	GA	AA
Cow Body Weight (kg)	546[a]	620[b]	541[a]

Table 7: relationship between G8398A polymorphisms and cow body weight [[ab] Least-squared means without a common superscript differ ($P < 0.05$)]

	G8398A Genotype		
	GG	GA	AA
Cow Hair Coat Score	2.5	3.3	2.9

Table 8: relationship between G8398A polymorphisms and cow hair coat score

Conclusions:

Based on the reported results showing the correlation between higher prolactin concentrations and lower hair coat scores (slicker hair coats) as well as greater body weights, it is believed that concentrations of circulating prolactin could be a useful predictor of cattle productivity.

Although, the various polymorphisms within the genetic markers studied (dopamine receptor A534G, melatonin receptor G497A, melatonin receptor A445G, prolactin coding sequence G8398A, prolactin promoter A1134T, and prolactin promoter C1286T) did not show a direct correlation with the recorded prolactin levels, both prolactin coding sequence polymorphism G8398A and prolactin promoter polymorphism A1134T showed a relationship with cow hair coat score and the G8398A polymorphism also showed a correlation with cow body weight. Based on this, it can be concluded that these two specific polymorphisms could be significant predictors in cow performance and productivity.